Towards an optimal TSH level: different goals for different outcomes and for different populations?

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CHAPTER 1

General introduction

Introduction

Case

A 74-year-old woman, living in an iodine deficient area, visits the general practitioner because of persistent fatigue. She has a medical history of dyslipidemia. One year ago, her thyroid function was tested because of fatigue, weight gain and cold intolerance. At that time, blood results showed a thyroid stimulating hormone (TSH) level of 3.8 mIU/L (reference range 0.4-4.0 mIU/L), a free thyroxine (FT_4) level of 10.2 pmol/L (reference range 8-22 pmol/L) and the presence of thyroid peroxidase antibodies. No other physical illness was revealed that might have caused the symptoms. Thyroid hormone substitution therapy was started. The symptoms of weight gain and cold intolerance improved slightly. However, despite the treatment with levothyroxine, she is still complaining of fatigue. Her current TSH level (while using levothyroxine 50 micrograms once daily) is 2.6 mIU/L.

This case raises several questions. Does this woman have thyroid dysfunction? Is the decision of the general practitioner to prescribe thyroid hormone replacement therapy a year ago justifiable? Would the age of the patient who presented with a TSH level of 3.8 mIU/L have influenced the decision on whether to start with replacement therapy or not? Should the clinician take other characteristics, such as the ethnicity or the (presumed) iodine status of the patient and her population background into account for the interpretation of the laboratory results and the decision on whether to initiate the replacement therapy? And once decided to start with replacement therapy, what should be the target level of TSH during treatment? Is the optimal target level of TSH the same for all patients? What is the cause of the persistent fatigue, despite adequate thyroid hormone replacement?

Clinicians, treating patients with thyroid dysfunction, have to deal with these issues frequently. Overt thyroid dysfunction is clearly associated with symptoms, morbidity and mortality. However, the consequences of small variations in thyroid function on symptoms, morbidity and mortality and the definition of normal thyroid function (i.e. the reference range of serum TSH) are still under debate. Currently, the reference range of serum TSH is based on the 2.5th and 97.5th percentile of the population. A more logical approach would be to base the reference limits on clinical outcomes. Therefore, studies investigating associations between thyroid function within the normal range and clinical outcomes are very relevant and needed. Apart from the reference limits of serum TSH, the optimal level of TSH to aim for during treatment (i.e. the target level) is not well known and this optimal TSH level could be different for each individual.

These crucial questions are at the core of this PhD project and serve as the basis on which the studies described in this thesis have been designed. The general aim of this thesis is to investigate short- and long-term effects of subtle differences in thyroid function and iodine status in the general population in order to provide new information that can be taken into account when 1) assessing the reference range of serum TSH and 2) to identify factors that should be taken into account when assessing the optimal TSH level for a patient during thyroid hormone replacement therapy.

This introductory chapter provides background information on thyroid function and iodine intake as well as an overview of previous studies regarding thyroid function within the normal range and iodine status in the general population. The research questions, resulting from this information, are formulated and the studies conducted will be introduced.

Thyroid function and thyroid function disorders

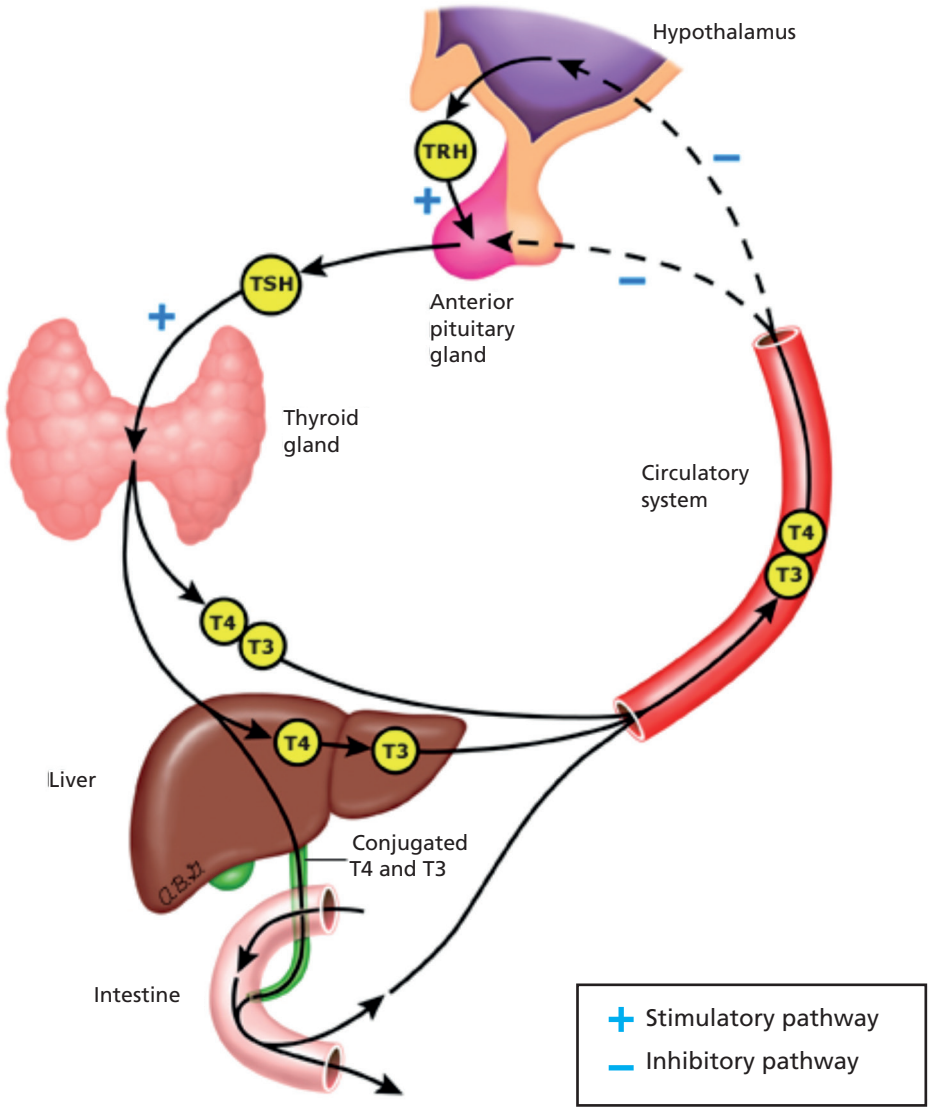
Background information on thyroid function

The thyroid gland produces the hormones thyroxine (T_4) and triiodothyronine (T_3), which exert effects virtually on all organ systems. Serum thyroid hormone levels are maintained within narrow limits by a regulatory mechanism that is very sensitive to small changes in circulating free hormone concentrations (figure 1). TSH, produced by the thyrotroph cells in the anterior pituitary gland, stimulates the thyroid gland to secrete T_4 and T_3. TSH production is controlled by thyrotropin-releasing hormone (TRH), which is produced in the parvocellular region of the paraventricular nuclei of the hypothalamus. TRH is distributed throughout the hypothalamus, but its content is highest in the median eminence and paraventricular nuclei. TSH secretion is down-regulated by the negative feedback of thyroid hormones (T_4 and T_3) on the hypothalamus and the pituitary gland (figure 1). Thyroid hormones are essential for the development and function of all organ systems, in particular the brain. Adequate synthesis of thyroid hormones requires on one hand a sufficient iodine intake and on the other hand the capacity of the thyroid gland to metabolize iodine and to incorporate it in organic compounds. Thyroid diseases are common in the general population, however the incidence of thyroid function disorders varies among different populations.[1-4]

Overt primary hypothyroidism is characterized by decreased serum free T_4 (FT_4) and increased serum TSH levels. An elevated serum TSH level associated with a normal FT_4 serum concentration is defined as subclinical primary hypothyroidism. In the eastern part

of the Netherlands, the prevalence of overt primary hypothyroidism and subclinical primary hypothyroidism is 0.4% and 4.0%, respectively.[4] The most common cause of primary hypothyroidism in iodine-sufficient areas is chronic autoimmune (Hashimoto's) thyroiditis. Iatrogenic causes such as thyroidectomy, radioiodine treatment, external radiation therapy and medications like amiodarone or lithium can also cause hypothyroidism. Central hypothyroidism is characterized by a low FT_4 serum concentration and a serum TSH concentration which is not appropriately elevated. It is caused by either hypothalamic (tertiary hypothyroidism) or pituitary (secondary hypothyroidism) disorders. Hypothyroidism causes a generalized reduction of the metabolic rate that results in symptoms like fatigue, cold intolerance, weight gain, constipation and musculoskeletal symptoms. Moreover, severe hypothyroidism can cause hypothermia, anemia, hyponatremia, increase of serum creatinine, bradycardia, decreased cardiac output, edema, pericardial effusion, hypertension, hypercholesterolemia, oligo- or amenorrhea, neurological dysfunction and myxedema coma.[5, 6] Overt hypothyroidism either primary or central requires replacement therapy with thyroid hormones.

Figure 1.
Thyrotropin-releasing hormone (TRH) increases the secretion of thyrotropin (TSH), which stimulates the synthesis and secretion of trioiodothyronine (T_3) and thyroxine (T_4) by the thyroid gland. T_3 and T_4 inhibit the secretion of TSH, both directly and indirectly by suppressing the release of TRH. T_4 is converted to T_3 in the liver and many other tissues by the action of T_4 monodeiodinases. Some T_4 and T_3 is conjugated with glucuronide and sulfate in the liver, excreted in the bile, and partially hydrolyzed in the intestine. Some T_4 and T_3 formed in the intestine may be reabsorbed. Reproduced with permission from: Ross DS. Thyroid hormone synthesis and physiology. In: UpToDate, Post TW (Ed), UpToDate, Waltham, MA. (Accessed on 1-4-2015). Copyright © 2015 UpToDate, Inc. For more information visit www.uptodate.com.



Overt primary thyrotoxicosis is characterized by increased serum FT_4 and decreased serum TSH levels. Subclinical primary thyrotoxicosis is characterized by a decreased serum TSH and a normal serum FT_4 level. In the eastern part of the Netherlands, the prevalence of overt primary thyrotoxicosis and subclinical primary thyrotoxicosis is 0.4% and 0.8%, respectively.[4] The most common causes of thyrotoxicosis are Graves' disease, multinodular toxic goitre, toxic adenoma and thyrotoxic phase of subacute thyroiditis. Thyrotoxicosis caused by increased TSH production by a pituitary tumor is rare. The classic symptoms of thyrotoxicosis are weight loss, heat intolerance, tremor, palpitations, anxiety, increased frequency of bowel movements and shortness of breath. Thyrotoxicosis can also cause increased cardiac output, hypertension, atrial fibrillation, osteoporosis and neuropsychiatric diseases.[5, 7] Different strategies can be applied for the treatment of overt thyrotoxicosis, including medication to inhibit thyroid hormone synthesis and/or release or methods that reduce the quantity of thyroid tissue (e.g. radioiodine or surgery).

Subclinical thyroid dysfunction can be associated with the same symptoms and diseases as overt thyroid dysfunction, although symptoms are generally less severe and the associations are generally less strong and more controversial as shown by the conflicting results of numerous studies.[8, 9] The benefit of treatment of subclinical thyroid dysfunction is even more debated, as several double-blind placebo-controlled studies have shown a lack of improvement of symptoms and outcomes in subclinical primary hypothyroidism.[8-10] At present, in the Netherlands, treatment of subclinical hypothyroidism is only recommended in the presence of symptoms of hypothyroidism.[11] Treatment of subclinical thyrotoxicosis is recommended in the presence of symptoms of thyrotoxicosis, osteoporosis, atrial fibrillation or other cardiac co-morbidity.[11]

Each individual has an own set-point of the hypothalamic-pituitary-thyroid axis.[12] Variation of serum TSH, (free) T_4 and T_3 levels within individuals is much smaller than between individuals. The width of the 95% confidence interval of thyroid hormone levels in an individual is approximately half that of the population.[12] Consequently, it is possible that a TSH level may be abnormal for an individual but still is within the laboratory reference limit.

For some time, lowering the upper limit of the reference range of serum TSH has been debated.[13-20] Reasons to question the present reference range of TSH are the high proportion of individuals whose serum TSH level is less than 2.5 mIU/L, the higher prevalence of thyroid autoantibodies in individuals with serum TSH level > 2.5 mIU/L and the observation that people with TSH levels between 2.5 and 4.5 mIU/L have an increased risk of progression to overt hypothyroidism.[13, 21] However, since about 20% of the subjects without known thyroid disease, without thyroid autoantibodies and with normal thyroid ultrasound has a TSH level between 2.5

mIU/L and 4.5 mIU/L and no firm evidence is available that lowering the upper limit of normal will provide any short- or long-term benefit for subjects, the upper limit of the reference range of TSH remained unchanged.[14, 16, 17] The question whether TSH level in the upper or lower part of the reference range is associated with clinical symptoms, cardiovascular risk factors, morbidity or mortality, is very important for the ongoing debate on narrowing the reference interval. In the past few years, several studies have investigated the relationship between thyroid function within the normal range and several outcomes.

Previous studies regarding thyroid function within the normal range and clinical outcome parameters

In the past few years, several studies have investigated the relationship between thyroid function within the reference range and several clinical outcomes including cardiovascular risk factors and morbidity, renal function and bone mineral density. Numerous previous studies examined the relationship between thyroid function within the normal range and cardiovascular risk factors, such as blood pressure, serum cholesterol, insulin sensitivity and adiposity. These studies reported conflicting results. Six studies found a positive association between TSH level within the normal range and systolic and/or diastolic blood pressure[22-27] and one study found a positive association between FT_4 level and systolic and diastolic blood pressure.[28] However, 10 studies found no association between thyroid function within the normal range and blood pressure at all.[29-38] The majority of the studies examining the association between thyroid function within the normal range and serum lipid levels found that low-normal thyroid function was associated with less favorable lipid concentrations[23, 28, 34, 35, 37-43], whereas 3 studies did not yield this association.[29, 31, 32] Five studies showed an association between thyroid function within the normal range and insulin resistance[28, 31, 35, 37, 44], but 3 other studies could not confirm this relationship.[29, 32, 34] Recently, the studies investigating the relationship between TSH within the normal range and adiposity were reviewed.[45] Of the 29 studies included in the review, 18 studies showed a positive relationship between measures of adiposity and serum TSH. In conclusion, of the cardiovascular risk factors, lipid concentration was most consistently associated with thyroid function within the normal range. The selected upper limit of TSH differed among all the studies, as well as the characteristics of the study population and the adjustment for potential confounders. These differences might explain the discrepancies of the study results.

Other studies investigated the relationship between thyroid function within the normal range and cardiovascular morbidity. Three large population-based studies found an association between high-normal thyroid function and atrial fibrillation.[46-48] One study showed that higher levels of TSH within the normal range were associated with an increased risk of myocardial

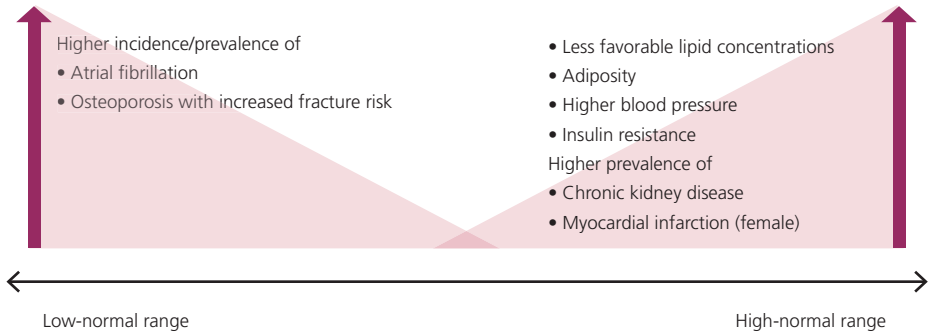
infarction in patients with clinically manifest vascular disease.[49]

Another large population-based study of 29,480 individuals (the HUNT study) investigated the relationship between the thyroid function within the normal range and renal function. In this study, TSH level within the reference range was negatively associated with the estimated glomerular filtration rate (eGFR) and the prevalence of chronic kidney disease (eGFR<60 ml/min/1.763m^2) was higher in individuals with low normal thyroid function.[50] This study was a cross-sectional survey, therefore no conclusion concerning causality could be made.

Several studies investigated the association between thyroid function within the normal range and bone mineral density (BMD) and these studies yielded conflicting results as well. Most of them showed an association between high-normal thyroid function and low BMD, osteopenia, osteoporosis or fracture risk.[51-56] By contrast, two large population-based studies found no relationship between thyroid function within the normal range and BMD.[57, 58] The discrepancy between these studies may be explained by the heterogeneity of the studies. There were differences in study population (clinical samples versus population-based studies), differences in reference limits of TSH, differences in measurement of BMD (lumbar spine versus hip or distal site of the forearm) and differences in adjustment for confounders.

Only few studies investigated the relationship between thyroid function within the normal range and mortality and these studies also reported conflicting results. Some studies showed that a high-normal thyroid function is positive associated with mortality.[59-62] Another study, comprising adults of 20 years or older, showed that higher TSH levels are associated with cardiovascular mortality in women.[63] Other studies showed no association at all between thyroid function within the normal range and mortality.[49, 64] The discrepancy of the results of previous studies investigating the association between thyroid function and mortality can be partially explained by the differences in study populations.

Figure 2.
Simplified overview of the relationship between serum TSH within the normal range and clinical parameters.



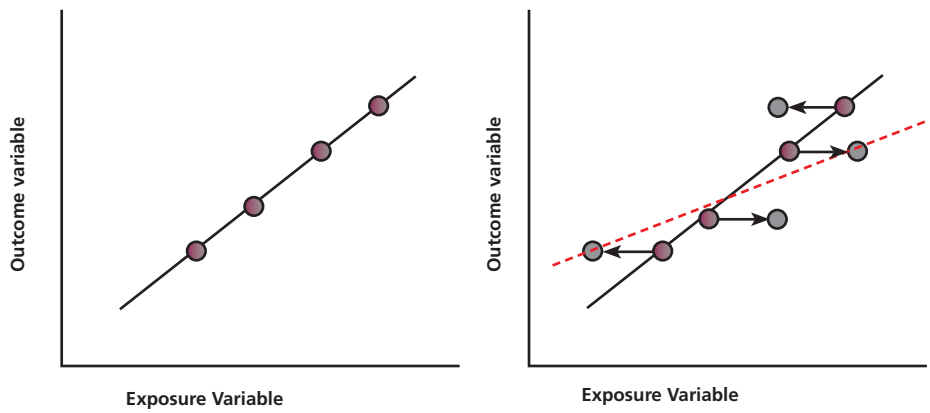
Research questions and conducted studies

As described above, several studies have investigated the relationship between thyroid function within the normal range and *cardiovascular risk factors*, *morbidity* and *mortality*. Data on the association between thyroid function within the normal range and the presence of *symptoms* are scarce. The first aim of this thesis was to investigate the association between thyroid function within the normal range and symptoms. Fatigue and depressive symptoms are frequently reported by patients with thyroid dysfunction and these are very relevant symptoms impairing quality of life of patients.[65, 66] We hypothesized that there is a relationship between thyroid function within the normal range and fatigue and depressive symptoms. The Nijmegen Biomedical Study (NBS) provided us the opportunity to test these hypotheses **(Chapter 2 and 3)**. The NBS is a large population-based survey undertaken in the eastern part of the Netherlands. A total of 22,451 age- and sex-stratified randomly selected adults received a questionnaire on lifestyle, medical history and symptoms (including the presence and severity of fatigue and depressive symptoms). Of each age-group (range 5 years), 750 men and 750 women were invited to participate. A total of 9,350 subjects, receiving a questionnaire, responded (response rate: 42%). Of the responders, 6,434 subjects (69%) gave permission for blood withdrawal and several items including thyroid hormones were measured in serum.

A second aim of this thesis was to investigate the relationship between thyroid function within the normal range and mortality, a long term outcome. Some previous studies suggest that age might influence the association between thyroid function and mortality.[67, 68] However, the total number of young participants and oldest elderly is limited in most studies. The NBS comprises randomly selected adults of all age groups. Therefore, this population-based study provided us the opportunity to investigate the influence of age on the relationship between thyroid function and mortality **(Chapter 4)**.

A factor that one should take into account when interpreting studies concerning the association between thyroid function and symptoms, diseases or mortality, is the variation of thyroid function. The overall variation of the thyroid function measurements consists of biological variation, preanalytical variation and analytical variation.[12, 69-71] The variation in thyroid function measurements blurs the association between thyroid function and the outcomes of interest (figure 3,[72]). This is called the regression dilution bias.[72-75] Studies, based on one single measurement of thyroid function, underestimate the association between thyroid function and symptoms, diseases and mortality. This is especially the case in studies with a long follow up period. The magnitude of underestimation of associations between several cardiovascular risk factors and cardiovascular diseases and mortality has been previously described.[73, 74, 76, 77] For the relationship between thyroid function and symptoms, diseases or mortality, the magnitude of this underestimation is still unknown. Therefore, a third aim of this thesis was to estimate the magnitude of underestimation of the relationship between thyroid function and symptoms, diseases and mortality due to variation of thyroid function. In order to determine the magnitude of this underestimation due to regression dilution bias, we used repeated measurements of thyroid function (with an interval of 2-4 years) in two large population-based surveys, the NBS and the Rotterdam Study **(Chapter 5)**.

Figure 3. The effect of variation of the exposure variable on the outcome variable. With no variation in measurement of the exposure variable (panel a), the slope of the line describes the error-free association between exposure variable and outcome variable. Variation of the exposure variable results in an attenuation of the slope (panel b) and the association between the exposure variable and outcome variable is underestimated.[72]



Iodine and thyroid function

Background information on iodine

Iodine is an essential micronutrient and an important component of thyroid hormones. The thyroid hormones T_4 and T_3 contain 4 and 3 iodine atoms, respectively. Iodine must be provided in the diet. Iodine deficiency can cause thyroid dysfunction, goiter and cretinism.[78] Iodine excess can also cause thyroid dysfunction. Monitoring the iodine status and maintaining an optimal iodine intake in the population is very important to prevent brain damage in newborns and thyroid function disorders at all ages. Iodine is a trace element in the crust of the earth and its distribution is quite variable. Many areas, particularly inland and mountainous regions, have minimal iodine concentrations in the crust of the earth, while others, often coastal regions, have sufficient or even excessive iodine concentrations. Soil and ground water (used for water supply systems) vary greatly in iodine content between different areas. Sea products and fish also contain iodine. Iodine deficiency remains a global public health problem.[79]

In the past, mild iodine deficiency was present in the eastern and southern part of the Netherlands.[80-82] Iodine supplements were instituted as of 1935. Since then, several additional measures, like the compulsory use of iodized salt in bakeries, instituted in 1963, were taken to achieve a daily intake of iodine within the optimal range as recommended by the WHO.[83] Currently, the iodine status of the Netherlands is considered to be adequate, based on studies regarding the iodine intake and urinary excretion of iodine in several regions in the Netherlands.[79, 84-88]

Previous studies regarding the relationship between iodine status and thyroid function

Previous population studies have shown that in populations with a history of mild or moderate iodine deficiency, the average TSH levels tend to decrease with age whereas FT_4 levels tend to increase with age, which is probably due to the gradual development of autonomous function of the thyroid gland.[4, 89, 90] In case of mild iodine deficiency, low iodine intake might lead to a reduced T_4 and T_3 production. In order to prevent this, several TSH-independent autoregulatory mechanisms within the thyroid are triggered, such as an increase in vascularity, an increase in iodine uptake and deiodination of T_4 to T_3.[91] If these mechanisms fail, TSH levels will rise in response to a lower thyroid hormone production. TSH stimulates follicular cell replication and due to the higher replication rate, the chance of activating mutations in the TSH-receptor gene leading to TSH-independent growth and function, is increased.[92] This and other mechanisms lead to autonomous function of the thyroid and, especially when iodine intake is supplemented, to hyperthyroidism. In subjects of the NBS, performed in Nijmegen, a municipality in the eastern part of the Netherlands with a history of mild iodine insufficiency, TSH level is negatively

associated with age and FT_4 is positively associated with age.[4] In contrast with the decrease of TSH level with age in populations with an iodine deficiency, TSH levels tends to increase with age in populations with high iodine intake.[3, 93-96]

Research questions and conducted studies

The previous population studies, showing a negative association between TSH levels and age in iodine insufficient areas, are cross-sectional studies. These findings raise the question whether such an age-thyroid function relation is apparent also in longitudinal analyses and, if so, whether it reflects an actual iodine deficiency. To answer this question, we analyzed longitudinal data on thyroid function and data on iodine status of participants of the NBS **(Chapter 6)**.

Previous studies showed that current differences in iodine intake influence the relationship between thyroid function and age. Because iodine insufficiency in the past might cause an ongoing increase of FT_4 and decrease of TSH levels even after attaining an adequate iodine status, we hypothesized that there also might be differences in the present relationship between thyroid function and age in case of differences in iodine intake in the past. Moreover, the relationship between thyroid function and age could be used as an indicator of present or historical iodine status. So, a further aim of this thesis was to investigate the effect of historical iodine status of a population on the current relationship between thyroid function and age. In order to test this hypothesis, we examined the relationship between thyroid function and age in several regions in the Netherlands which are iodine sufficient at present but with a difference in iodine intake in the past **(Chapter 7)**.

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CHAPTER 2

Is there a relationship between fatigue perception and the serum levels of thyrotropin and free thyroxine in euthyroid subjects?

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Abstract

Background

Thyrotoxicosis and hypothyroidism are associated with fatigue. Here we studied euthyroid subjects to determine if there was a relationship between serum thyroid stimulating hormone (TSH), free thyroxine (FT_4) and thyroperoxidase antibodies and fatigue.

Methods

A total of 5,897 participants of the Nijmegen Biomedical Study received a questionnaire and serum TSH (normal range 0.4-4.0 mIU/L) and FT_4 (normal range 8-22 pmol/L) were measured. Fatigue was evaluated by the RAND-36 and the Shortened Fatigue Questionnaire (SFQ).

Results

Euthyroid subjects with a serum TSH level of 0.4-1.0 mIU/L had a lower RAND-36 vitality score (65.2 versus 66.8, regression coefficient (RC) -1.6, 95% CI -2.6 ; -0.5, p-value 0.005) and a higher SFQ score (11.7 versus 11.0, RC 0.6, 95% CI 0.2 ; 1.0, p-value 0.004) than those with a TSH of 1.0-2.0 mIU/L. Those with a serum FT_4 of 18.5-22 pmol/L reported fatigue more often (52.5% versus 33.3%, relative risk (RR) 1.4, 95% CI 1.0 ; 1.9, p-value 0.03), had a lower RAND-36 vitality score (61.7 versus 66.6, RC -4.4, 95% CI -8.1 ; -0.6, p value 0.02) and a higher SFQ score (13.2 versus 11.0, RC 1.9, 95% CI 0.4 ; 3.3, p-value 0.01) than subjects with a FT_4 level of 11.5-15 pmol/L. In comparison to euthyroid subjects without known thyroid disease, euthyroid subjects with previously known thyroid disease reported fatigue more often (52.3% versus 34.0%, RR 1.3, 95% CI 1.0 ; 1.5, p-value 0.025), had a lower RAND-36 vitality score (61.4 versus 66.3, RC -2.9, 95% CI -5.3 ; -0.6, p-value 0.015) and a higher SFQ score (13.7 versus 11.1, RC 1.4, 95% CI 0.5 ; 2.3, p-value 0.002).

Conclusion

In euthyroid individuals without a history of thyroid disease there is a modest relationship between thyroid function and fatigue with subjects having apparently higher production of T_4 experiencing more fatigue. Subjects with a history of thyroid disease, but with normal TSH and FT_4 concentrations, experience more fatigue than the general population. The reasons for this are unclear but subtle abnormalities in the dynamics of thyroid hormone secretion should be considered.

Introduction

Thyroid dysfunction is common in the general population.[1-4] Fatigue is a frequently reported symptom of patients with both hyper- and hypothyroidism.[5-9] Fatigue is also one of the most relevant symptoms for patients with thyroid dysfunction impairing their quality of life.[9, 10] As for mild degrees of thyroid dysfunction, Canaris *et al*. reported more symptoms, including feeling more tired, in subclinically hypothyroid subjects in comparison to euthyroid subjects in a large population-based study.[2] In contrast, Grabe *et al*. found no increase of fatigue in subjects with subclinical and overt hypo- or hyperthyroidism in a population-based study.[11]

In the absence of suitable methods for measuring thyroid hormone and thyroid stimulating hormone (TSH) production rates in groups of patients, by generally accepted definition, subjects with serum TSH and free thyroxine (FT_4) concentrations within the normal range are considered to be euthyroid. Previous studies have shown an association between variations in serum TSH and FT_4 concentrations within the normal range and cardiovascular risk, blood pressure, body mass index, incidence of atrial fibrillation and serum cholesterol.[12-17] These results raise the question of whether there is also an association between fatigue and serum TSH and FT_4 within the normal range. Recently, an association between the level of thyroperoxidase antibodies (TPOAbs) and fatigue has been described in euthyroid women with a goiter.[18] This raises the question whether TPOAb levels are associated with fatigue in the general population.

The present study had several goals. The first was to investigate whether unsuspected thyroid dysfunction (i.e. overt or subclinical thyrotoxicosis or overt or subclinical hypothyroidism) as uncovered in a population, was associated with the prevalence and severity of fatigue. The second was to investigate whether, in subjects whose serum TSH and FT_4 are in the normal range, there is a relationship between the levels of serum TSH or serum FT_4 and the prevalence and severity of fatigue. The last was to determine in apparently euthyroid subjects (i.e. normal TSH and FT_4) whether there is an association between the presence of TPOAbs and fatigue.

Methods

Study participants

The subjects of this study were participants of the Nijmegen Biomedical Study (NBS), a large, population-based survey performed in Nijmegen, a town in the eastern part of The Netherlands. Details of this study have been described previously.[4] Approval to conduct the study was obtained from the Institutional Review Board. A total of 22,451 age- and sex-stratified randomly selected adults received a questionnaire on lifestyle, medical history and symptoms. Of each age-group (range 5 years), 750 men and 750 women were invited to participate. We excluded pregnant women and subjects using medication interfering with thyroid function such as lithium, amiodarone, kelp, oral glucocorticosteroids and/or dopamine agonists because of the possible effect of these conditions and medications on thyroid function. In order to investigate the population without known thyroid disease, we excluded the subjects with previously known thyroid disease, subjects with the use of thyromimetic and/or thyrostatic drugs, and subjects with a history of thyroid surgery and/or radioactive iodine treatment.

Laboratory methods

Serum TSH was measured by an immunoluminometric assay on a random-access analyzer (Architect; Abbott Diagnostics Division). The reference interval used in our laboratory is 0.4-4.0 mIU/L. Serum FT_4 was measured with a luminescence enzyme immunoassay on a random-access assay system (Vitros ECI; Ortho Clinical Diagnostics). Our laboratory reference interval is 8.0-22.0 pmol/L. Antibodies against TPO (TPOAbs) were measured with a fluorescence immunoenzymometric assay for the quantitative measurement of the IgG class of anti-thyroperoxidase antibodies (AxSYM Anti-TPO; Abbott Diagnostics Division). The reference interval was defined as <12 kIU/L (data provided by manufacturer). More details about these measurements are described elsewhere.[4] Thyroid function was classified as overt thyrotoxicosis if TSH was <0.4 mIU/L and FT_4 was >22 pmol/L and it was classified as subclinical thyrotoxicosis if TSH was <0.4 mIU/L and FT_4 was ≥8 pmol/L and ≤ 22 pmol/L. Thyroid function was classified as overt hypothyroidism if TSH was >4.0 mIU/L and FT_4 was <8 pmol/L and as subclinical hypothyroidism if TSH was >4.0 mIU/L and FT_4 was ≥8 pmol/L and ≤ 22 pmol/L. When both TSH and FT_4 were within normal range, thyroid function was classified as euthyroidism. When either TSH or FT_4 was not within the normal range, thyroid function was classified as thyroid dysfunction. Subjects with overt thyrotoxicosis, subclinical thyrotoxicosis, overt hypothyroidism, or subclinical hypothyroidism were considered to have thyroid dysfunction.

Questionnaire

The questionnaire contained questions about gender, age, weight, height, lifestyle, medical history and the use of medication. The presence of the symptom fatigue was evaluated by the question: 'Do you feel tired?'. Data on the presence of the symptom fatigue were missing in 119 subjects. The subscale vitality (energy and fatigue) of the RAND-36 Item Health Survey 1.0 was used to determine the intensity of fatigue1.[9] The items of the subscale vitality are identical to the MOS SF-36.[20] The subscale vitality of the RAND-36, translated in Dutch, has a good internal reliability (Cronbachs α: 0.82).[21, 22] It consists of four questions: 'How much of the time during the past 4 weeks...- did you feel full of life? - did you have a lot of energy? - did you feel worn out? - did you feel tired?'. The participants choose the best option of the 6 possible answers given for each question: 'all of the time', 'most of the time', 'a good bit of the time', 'some of the time', 'a little of the time', or 'none of the time'. All items were scored on a 0 to 100 range and recoded so, that a high score defined a more favourable health state. The average score of the 4 questions was calculated. When 1 or 2 items were missing, the average score of the other 2 or 3 questions was used. If 3 or 4 items were missing, the total score was defined as missing and not used for the analyses. The score of the subscale vitality of the RAND-36 was missing in 178 subjects. A higher score is associated with having more energy and being less fatigue, with a maximum score of 100. The average score of the subscale vitality of the RAND-36 in a Dutch cohort, consisting of 1063 subjects, aged 18-89 years, was 67.4 (SD 19.9).[22] In this cohort, young adults (19-24 years old) scored on average 69.2 points, whereas elderly (75-85 years old) scored on average 60.1 points.

In addition, the Shortened Fatigue Questionnaire (SFQ) was also used to determine the intensity of the fatigue.[23, 24] The SFQ is a short and easy to use instrument to determine the intensity of fatigue. The SFQ has a good internal reliability (Cronbach-α: 0.88) and discriminating validity. It consists of four statements: "I feel tired", "I tire easily", "I feel fit", and "I feel physically exhausted". The participants rated the statements for the degree of being true at a 7-point scale and for each answer points were granted. A higher score is associated with being more tired, with a maximum score of 28 points. Healthy adults, without any stressful conditions, score on average 5-8 points, whereas for example patients with cancer score on average 13-21 points.[23] The SFQ score was missing in 497 subjects.

Statistical analysis

The body mass index (BMI) was calculated by dividing the body weight (kg) by the square of the height (m). Relative risks along with the 95% confidence intervals (CI) were estimated using loglinear regression analyses, with fatigue as the dependent variable and thyroid function class and TSH and FT_4 subclasses within the normal range as the independent variables.[25-27] Also the

presence of TPOAbs (defined as >12kIU/mL according to the reference interval provided by the manufacturer) and the presence of known thyroid disease were used as independent variables in the loglinear analysis.

In order to compare the score of the vitality subscale of the RAND-36 and the SFQ score of each thyroid function class, we used linear regression analyses with the fatigue score as the dependent variable and thyroid function class as the independent variable. Within the normal range of thyroid function, we used subclasses of TSH and FT_4 for linear regression analyses with the score of the subscale vitality of the RAND-36 or the SFQ score as the dependent variable and the TSH subclasses and FT_4 subclasses as the independent variables. The presence of TPOAb levels was also used as the independent variables for linear regression analyses with the score of the subscale vitality of the RAND-36 or the SFQ score as the dependent variable.

All regression analyses were adjusted for gender, age, BMI and smoking status in order to eliminate possible confounders. In addition, we adjusted for a medical history of chronic obstructive pulmonary disease (COPD)/asthma, rheumatoid disease, cardiovascular disease (CVD), cancer, diabetes mellitus, kidney disease, liver disease and C-reactive protein (CRP) to rule out the possible confounding effect of non-thyroidal illness. We analysed the data with STATA version 11.0 (StataCorp, Texas).

Results

A total of 9350 subjects, receiving a questionnaire, responded (response rate: 42%). Of the responders, 6434 subjects (69%) gave permission for blood withdrawal. Of the responders, the subjects who gave permission for blood withdrawal differed only slightly from the subjects who did not donate blood samples: the mean age was 56 versus 53 years respectively, the percentage of women was 54% versus 50%, the prevalence of fatigue was 36% versus 37%, the mean RAND-36 vitality score was 65.7 versus 63.9 and the SFQ score was 11.4 versus 12.2. We excluded 47 pregnant women and 162 subjects using medication interfering with thyroid function. In addition, we excluded 328 subjects because of previously known thyroid disease.

Table 1.
Characteristics of the population.

Characteristic	Total n=5897		Female n=3101		Male n=2796	
Age (years)	55.6	± 17.9	53.1	± 18.1	58.5	± 17.2
BMI (kg/m^2)	25.2	± 4.1	24.8	± 4.5	25.6	± 3.6
Smoking (n %)	1327	(22.5%)	652	(21.0%)	675	(24.1%)
Medical history of:						
COPD/asthma (n %)	725	(12.3%)	375	(12.1%)	350	(12.5%)
CVD (n %)	586	(9.9%)	161	(5.2%)	425	(15.2%)
rheumatic disease (n %)	502	(8.5%)	329	(10.6%)	173	(6.2%)
cancer (n %)	453	(7.7%)	229	(7.4%)	224	(8.0%)
diabetes mellitus (n %)	321	(5.4%)	145	(4.7%)	176	(6.3%)
kidney disease (n %)	173	(2.9%)	87	(2.8%)	86	(3.1%)
liver disease (n %)	138	(2.3%)	79	(2.5%)	59	(2.1%)
CRP > 10 mg/L (n %)	544	(9.2%)	290	(9.4%)	254	(9.1%)
Fatigue (n %)	1989	(34.4%)	1195	(39.5%)	794	(28.9%)
RAND-36 score	66.2	± 17.4	64.1	± 17.3	68.6	± 17.3
SFQ score	11.2	± 6.4	12.0	± 6.6	10.3	± 6.1
TSH (mIU/L)	1.4	(0.9-2.0)	1.4	(0.9-2.0)	1.4	(0.9-1.9)
FT_4 (pmol/L)	13.3	(12-14.6)	13.3	(12-14.6)	13.3	(12-14.7)
TPOAbs positive (n %)	747	(12.7%)	520	(16.8%)	227	(8.1%)
Euthyroid (n %)	5439	(92.2%)	2823	(91.0%)	2616	(93.6%)

Data are reported as number (percent), mean ± standard deviation, or median (IQR).
BMI, body mass index; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; CRP, C-reactive protein; SFQ, shortened fatigue questionnaire; TSH, thyrotropin; FT_4, free thyroxine; TPOAbs, thyroperoxidase antibodies.

The characteristics of the remaining 5897 subjects are shown in Table 1. The age ranged from 18 years until 98 years. Female gender, a higher age, a higher BMI and a medical history of cardiovascular disease, cancer, diabetes, rheumatoid arthritis, asthma/COPD, renal disease, liver disease and a high CRP were all associated with a higher prevalence of fatigue.

Table 2 shows the prevalence of self-reported fatigue, the RAND-36 vitality subscale score and SFQ score according to the thyroid function class. Subjects with thyroid dysfunction reported fatigue more frequently (39.6%) than euthyroid subjects (34.0%), but this difference was not statistically significant. The relative risk was 1.2 (95% confidence interval (CI) 0.996 ; 1.4, p-value 0.055). After adjustment for possible confounders, the relative risk was 1.1 (95% CI 0.9 ; 1.3, p-value 0.34). There was no statistically significant difference in RAND-36 score or SFQ score between euthyroid subjects and subjects with thyroid dysfunction, neither before nor after adjustment for possible confounders.

Table 2.
Self-reported fatigue and scores of the RAND-36 vitality subscale and SFQ by thyroid function class, in subjects free of known thyroid disorder (N=5897).

Subjects		Self-reported fatigue		RAND-36 vitality		SFQ	
	n	%	RR (CI)	Score	RC (CI)	Score	RC (CI)
Euthyroidism	5439	34.0	Reference	66.3	Reference	11.1	Reference
Thyroid dysfunction[a]	458	39.6	1.1 (0.9 ; 1.3)	65.4	-0.3 (-1.9 ; 1.4)	11.6	0.1 (-0.5 ; 0.7)
- Overt thyrotoxicosis	9	55.6	1.6 (0.7 ; 3.9)	63.9	-1.9 (-12.8 ; 9.0)	13.8	2.4 (-1.5 ; 6.3)
- Subclinical thyrotoxicosis	196	37.0	1.0 (0.8 ; 1.3)	65.0	-0.8 (-3.3 ; 1.7)	11.9	0.4 (-0.5 ; 1.3)
- Subclinical hypothyroidism	216	42.8	1.1 (0.9 ; 1.4)	66.0	0.3 (-2.1 ; 2.7)	11.2	-0.3 (-1.2 ; 0.6)
- Overt hypothyroidism	22	28.6	0.8 (0.4 ; 1.8)	67.6	1.7 (-5.5 ; 8.8)	11.5	0.2 (-2.5 ; 2.9)

The relative risks (RRs) and regression coefficients (RCs) with 95% confidence interval (CI) were adjusted for gender, age, body mass index, smoking status and co-morbidity.

[a]Of the subjects with thyroid dysfunction, 7 subjects had a serum TSH >0.4 mIU/L and FT_4 >22 pmol/L and 8 subjects had a serum TSH <4.0 mIU/L and FT_4 <8 pmol/L.

Table 3.
Self-reported fatigue and scores of the RAND-36 vitality subscale and SFQ in euthyroid subjects, free of known thyroid disorder, by TSH and FT_4 within the normal range (N=5439).

Subjects			Self-reported fatigue		RAND-36 vitality		SFQ	
		n	%	RR (CI)	Score	RC (CI)	Score	RC (CI)
TSH	0.4-1.0 mIU/L	1493	36.2	1.1 (0.97 ; 1.2)	65.2	-1.6 (-2.6 ; -0.5)[a]	11.7	0.6 (0.2 ; 1.0)[a]
TSH	1.0-2.0 mIU/L	2754	33.3	reference	66.8	reference	11.0	reference
TSH	2.0-3.0 mIU/L	918	31.7	1.0 (0.8 ; 1.1)	66.9	0.4 (-0.9 ; 1.7)	10.7	-0.4 (-0.9 ; 0.1)
TSH	3.0-4.0 mIU/L	274	36.3	1.1 (0.9 ; 1.4)	65.7	-0.8 (-2.9 ; 1.3)	11.1	0.0 (-0.8 ; 0.8)
FT_4	8.0-11.5 pmol/L	808	33.4	1.0 (0.9 ; 1.2)	66.4	-0.1 (-1.5 ; 1.2)	11.1	0.0 (-0.5 ; 0.5)
FT_4	11.5-15 pmol/L	3517	33.3	reference	66.6	reference	11.0	reference
FT_4	15-18.5 pmol/L	1023	35.3	1.0 (0.9 ; 1.1)	65.7	-0.3 (-1.5 ; 0.9)	11.5	0.2 (-0.3 ; 0.6)
FT_4	18.5-22 pmol/L	91	52.2	1.4 (1.0 ; 1.9)[a]	61.7	-4.4 (-8.1 ; -0.6)[a]	13.2	1.9 (0.4 ; 3.3)[a]

The RRs and RCs (CI) were adjusted for gender, age, body mass index, smoking status and co-morbidity.
[a] p-value<0.05.

The prevalence of fatigue, the score of the subscale vitality of the RAND-36 and the SFQ score by TSH and FT_4 subclasses within the normal range (i.e. in euthyroid subjects) are shown in table 3. Within the normal range of TSH, subjects with a TSH level of 0.4-1.0 mIU/L had a lower RAND-36 vitality score (65.2 versus 66.8, mean difference -1.6, 95% CI -2.6 ; -0.5, p-value 0.005) and a higher SFQ score (11.7 versus 11.0, mean difference 0.6, 95% CI 0.2 ; 1.0, p-value 0.004) than those with a serum TSH of 1.0 to 2.0 mIU/L, after adjustment for possible confounders. Subjects with a serum FT_4 level of 18.5 to 22 pmol/L reported fatigue more often (52.5% versus 33.3%, relative risk 1.4, 95% CI 1.0 ; 1.9, p-value 0.03), had a lower RAND-36 vitality score (61.7 versus 66.6, mean difference -4.4, 95% CI -8.1 ; -0.6, p value 0.02) and a higher SFQ score (13.2 versus 11.0, mean difference 1.9, 95% CI 0.4 ; 3.3, p-value 0.01) than subjects with a serum FT_4 level of 11.5 to 15 pmol/L. Of the 1493 subjects with a TSH level between 0.4 and 1.0 mIU/L, 2.7% of the subjects (40 subjects) had a FT_4 level between 18.5 and 22 pmol/L. These 40 subjects reported fatigue more often (58% versus 36%, RR 1.6, 95% CI 1.2 ; 2.2, p-value 0.001), had a (not statistically significant) lower RAND-36 vitality subscale score (62.2 versus 65.2, RC -3.2, 95% CI -9.0 ; 2.5, p-value 0.27) and a higher SFQ score (13.6 versus 11.6, RC 2.2, 95% CI 0.1 ; 4.3, p-value 0.04) than subjects with a TSH level between 0.4 and 1.0 mIU/L and a FT_4 level between 8 and 18.5 pmol/L. Of the 91 subjects with a FT_4 level between 18.5 and 22 pmol/L, 44.0% of the subjects (40 subjects) had a TSH level between 0.4 and 1.0 mIU/L. These 40 subjects did not differ from the 51 subjects with a TSH level between 1.0 and 4.0 mIU/L in self-reported fatigue, RAND-36 vitality subscale score and SFQ score (self-reported fatigue 58% versus 48%, RR 1.4, 95% CI 0.9 ; 2.1, p-value 0.11; RAND-36 vitality subscale score 62.2 versus 61.3, RC -0.2, 95% CI -7.6 ; 7.3, p-value 0.97; SFQ score 13.6 versus 12.8, RC 1.5, 95% CI -1.3 ; 4.3, p-value 0.30). There were no differences in gender, BMI, smoking status or co-morbidity between the subclasses of TSH and FT_4. However, subjects with a TSH level of 0.4-1.0 mIU/L and subjects with a serum FT_4 level of 18.5 to 22 pmol/L were older (mean age 57.9 and 67.2 years respectively) than the reference group (mean age 54.6 and 54.1 years respectively). Subanalyses using different age groups gave similar results regarding the association between the subclasses of TSH and FT_4 levels within the normal range and fatigue (self-reported fatigue, RAND-36 vitality subscale score and SFQ score) (data not shown).

There was no association between the prevalence of self-reported fatigue, the RAND-36 vitality subscale score or the SFQ score and the presence of TPOAbs, neither in euthyroid subjects nor in subjects with thyroid dysfunction (table 4). Subdividing the subjects with TPOAbs according to the level of TPOAbs did not change these results (data not shown).

When comparing the study population with the subjects, excluded because of previously known thyroid disorder, subjects with a known thyroid disorder reported fatigue more often (50%

versus 34.4%, RR 1.2, 95% 1.0 ; 1.4, p-value 0.03), had a lower RAND-36 vitality score (61.8 versus 66.2, mean difference -2.6, 95% CI -4.5 ; -0.6, p-value 0.01) and a higher SFQ score (13.5 versus 11.2, mean difference 1.3, 95% CI 0.6 ; 2.1, p-value<0.001) than subjects without known thyroid disorder (table 5). Even in subjects who had serum TSH and FT_4 concentrations in the normal range, those with previously known thyroid disease reported fatigue more often (52.3% versus 34.0%, relative risk 1.3, 95% CI 1.0 ; 1.5, p-value 0.025), and had a lower RAND-36 vitality score (61.4 versus 66.3, mean difference -2.9, 95% CI -5.3 ; -0.6, p-value 0.015) and a higher SFQ score (13.7 versus 11.1, mean difference 1.4, 95% CI 0.5 ; 2.3, p-value 0.002). The median TSH of euthyroid subjects without previously known thyroid disease was 1.4 mIU/L (interquartile range (IQR) 1.0-1.9). The median TSH of subjects with known thyroid disease and TSH levels within normal range did not differ: 1.4 mIU/L (IQR 0.9-2.1). The median FT_4 of euthyroid subjects without previously known thyroid disease was 13.3 pmol/L (IQR 12.1-14.6). The median FT_4 of subjects with previously known thyroid disease and serum TSH and FT_4 within the normal range was 14.3 pmol/L (IQR 12.5-15.9). This differed significantly from the FT_4 levels of euthyroid subjects without previously known thyroid disease (RC 0.80, 95% CI 0.5 ; 1.1, p-value<0.05 after adjustment for possible confounders).

Table 4.
Self-reported fatigue and scores of the RAND-36 vitality subscale and SFQ by TPOAb subclasses in subjects free of known thyroid disorder (N= 5897).

Subjects		Self-reported fatigue			RAND-36 vitality		SFQ	
		n	%	RR (CI)	Score	RC (CI)	Score	RC (CI)
Total population								
TPOAbs	negative	5150	34.3	reference	66.3	reference	11.2	reference
TPOAbs	positive	747	35.3	1.0 (0.8 ; 1.1)	66.0	0.5 (-0.9 ; 1.8)	11.3	-0.1 (-0.6 ; 0.4)
Euthyroid subjects								
TPOAbs	negative	4870	33.9	reference	66.3	reference	11.1	reference
TPOAbs	positive	569	34.6	1.0 (0.8 ; 1.1)	66.3	0.7 (-0.9 ; 2.2)	11.4	0.1 (-0.5 ; 0.7)
Subjects with thyroid dysfunction								
TPOAbs	negative	280	48.9	reference	65.6	reference	12.0	reference
TPOAbs	positive	178	42.1	0.8 (0.6 ; 1.2)	65.2	0.5 (-3.0 ; 4.0)	11.1	-1.2 (-2.5 ; 0.1)

The RRs and RCs (with CI) were adjusted for gender, age, body mass index, smoking status and co-morbidity.

Table 5.
Prevalence of fatigue and scores of the RAND-36 vitality subscale and SFQ by medical history of thyroid disease.

Subjects		Self-reported fatigue		RAND-36 vitality		SFQ	
	n	%	RR (CI)	score	RC (CI)	score	RC (CI)
Total population							
Without previously known thyroid disorder	5897	34.4	reference	66.2	reference	11.2	reference
With previously known thyroid disorder	328	50.0	1.2 (1.0 ; 1.4)[a]	61.8	-2.6 (-4.5 ; -0.6)[a]	13.5	1.3 (0.6 ; 2.1)[a]
Euthyroid subjects							
Without previously known thyroid disorder	5439	34.0	reference	66.3	reference	11.1	reference
With previously known thyroid disorder	221	52.3	1.3 (1.0 ; 1.5)[a]	61.4	-2.9 (-5.3 ; -0.6)[a]	13.7	1.4 (0.5 ; 2.3)[a]
Subjects with thyroid dysfunction							
Without previously known thyroid disorder	458	39.6	reference	66.4	reference	11.6	reference
With previously known thyroid disorder	107	45.1	1.1 (0.8 ; 1.5)	62.6	-1.9 (-5.8 ; 1.9)	13.2	1. (-0.1 ; 2.8)

The RRs and RCs (with CI) were adjusted for gender, age, body mass index, smoking status and co-morbidity.
[a]p-value <0.05

Discussion

In this cross-sectional population study, we found an association between the presence and severity of fatigue and low-normal serum TSH levels and high-normal FT_4 levels. Although the magnitude of the differences was small and of dubious clinical significance, these results raise the question whether a serum TSH-value in the middle- or high-normal range would represent an optimal thyroid function. Indeed, the Rotterdam study has shown that a high-normal serum TSH level is associated with a lower risk of atrial fibrillation.[16] However, other previous studies have shown a higher risk of cardiovascular mortality, an association with a higher blood pressure, a higher body mass index and a higher non-HDL cholesterol in subjects with serum TSH-values within the high-normal range.[12-15, 17] These findings suggest that there may be different optimal serum TSH levels for each medical condition and symptom.

Previous studies have reported conflicting results regarding the association of thyroid function and fatigue in the general population. Canaris et al. reported more symptoms, including an increase of tiredness in subclinically and overtly hypothyroid subjects in comparison to euthyroid subjects in the Colorado study.[2] The Colorado thyroid disease prevalence study was a large, cross-sectional population study that examined the prevalence of abnormal thyroid function by measuring the serum TSH and FT_4 in 25,862 participants in a statewide health fair in Colorado in 1995. Besides the prevalence of thyroid dysfunction, the relationship between abnormal thyroid function and symptoms was examined, among which the symptom of feeling more tired. Due to the larger study population, this study comprised more subclinically and overtly hypothyroid subjects in comparison to our study. Also, the symptom feeling more tired was evaluated as an increase of tiredness over time instead of tiredness as a current symptom. This may explain the discrepancy with our results with respect to the prevalence of fatigue in patients with overt or subclinical hypothyroidism. Another explanation is that the participants of our study were not aware of the fact that thyroid function was tested and therefore this could not have affected their response to the questionnaire. Grabe *et al.* found no increase in mental or physical complaints, including fatigue, in subjects with subclinical or overt hypo- or hyperthyroidism in a population study with 3790 participants of the Study of Health in Pomerania (SHIP). Interestingly, subjects with overt or subclinical hyperthyroidism seemed to have less complaints in comparison to euthyroid subjects, but the prevalence of the symptom fatigue did not differ significantly.[11] These results are in accordance with the results in our study. Neither of the previous studies examined the association between TSH and FT_4 within the normal range and fatigue or used a validated test to establish the severity of fatigue.

In contrast with the association we found between the presence and severity of fatigue and low-normal TSH and high-normal FT_4 levels, we could not detect an obvious association between overt or subclinical hypo- or hyperthyroidism and fatigue. This is also in contrast with the high prevalence of fatigue in patients with known thyroid dysfunction.[5-7] One explanation may be that if thyroid dysfunction has led to complaints like tiredness, it is likely that these subjects have sought medical care and received therapy. These subjects were excluded from our analyses. This may has led to an underestimation of the association between thyroid dysfunction and fatigue in our study. Another explanation may be that thyroid dysfunction of the subjects in this study is less severe than that of the population consisting of referred patients. A third explanation might be that in our study, only a single blood sample was obtained for determining the thyroid hormone levels. Thyroid dysfunction can be transient and normalize spontaneously. These subjects might be less likely to complain of fatigue.

However, based on our data we believe that this is not the only explanation for this discrepancy. The prevalence of fatigue in subjects with thyroid dysfunction in our study was 39.6%, only slightly higher than the prevalence of fatigue in euthyroid patients, which was 34.0%. Because of the high prevalence of both fatigue and thyroid (subclinical) dysfunction in the general population, it is likely that many patients who seek medical care because of fatigue, happen to be tired and by coincidence also have thyroid dysfunction, without any causal relationship. Patients with fatigue are more likely to have their thyroid status tested by the general practitioner and thyroid dysfunction will be found more often, despite the absence of a causal relationship. This kind of selection bias is called confounding by indication.[28] Theoretically, treatment of thyroid dysfunction in these cases would not resolve the symptom fatigue. Although it is difficult to extend these findings of an epidemiological study to individual patients, we hypothesize that this might be the reason why in clinical practice, a subset of patients with thyroid dysfunction still complains of fatigue, despite optimal treatment and despite achieving euthyroidism. Our finding that subjects with known thyroid disease reported fatigue more often, had a lower RAND-36 vitality score and had a higher SFQ score, despite normal serum TSH and FT_4 levels, supports this hypothesis. This hypothesis is also compatible with previous randomized controlled studies in which treatment of subclinical hypothyroidism and subclinical thyrotoxicosis had no effect on health related quality of life and symptoms.[29, 30]

Confounding by indication is one explanation for the finding that subjects with known thyroid disease reported fatigue more often, regardless of normal serum TSH and FT_4 levels. Another explanation might be that the underlying disease, like chronic autoimmune thyroiditis, Graves' disease or goiter, may cause fatigue regardless of the presence of thyroid dysfunction or is associated with other diseases which may cause fatigue as well. Also, the fact that subjects

are aware of having thyroid disease may influence their feeling of well-being and have an impact on their quality of life, which may result in a higher fatigue score. In addition, subtle abnormalities in the dynamics of thyroid hormone secretion, even within the normal range, should be considered; the FT_4 levels of subjects with known thyroid disease and with thyroid hormone levels within the normal range were slightly higher in comparison to euthyroid subjects without known thyroid disease. Similar to our results, Engum et al. have reported an association between previously known thyroid disease and depression and anxiety, independent of thyroid function, in a large population based survey.[31]

We found no association between the level of TPOAbs and fatigue. This is in contrast with the recently described association between TPOAbs and both the presence of fatigue and the vitality subscale score of the SF-36 questionnaire.[18] Perhaps the difference in study population is the cause of this discrepancy. The study of Ott et al. comprised euthyroid women with a benign goiter. Our study was population-based, comprising both men and women, without known thyroid disease.

Our study has some limitations. Because of the cross-sectional observational nature of our study, no causal relationship or lack of causal relationship can be determined. Second, the results in the group of patients with overt hypothyroidism or thyrotoxicosis should be interpreted cautiously, as only a small number of participants were found to have an overt thyroid dysfunction. We did not measure T_3, so we might have missed some cases of overt thyrotoxicosis in subjects with normal FT_4 and elevated T_3 and misclassified those subjects as having a subclinical thyrotoxicosis. Third, antibodies against thyroglobulin were not measured in this epidemiological survey, so we might have missed some cases of autoimmune thyroiditis.[32] Fourth, despite the fact that the subscale vitality of the RAND-36 and the SFQ we used have been validated as excellent tools to screen for the presence and severity of fatigue, these instruments have only a limited value with respect to their ability to differentiate and quantify all the dimensions of fatigue. It is difficult to establish the clinical meaningfulness of a 1 point higher score on a fatigue scale. Finally, we cannot rule out a selection bias due to a difference in fatigue or thyroid function in the responders group versus the non-responders group and we cannot rule out the presence of other, unmeasured possible confounders.

In conclusion, within the normal range of TSH and FT_4, fatigue seemed more severe in subjects with low-normal TSH levels and high-normal FT_4 levels than middle-normal TSH and FT_4 levels, although the effect was small. Subjects with a history of thyroid disease, but with normal TSH and FT_4 concentrations, experience more fatigue than the general population. The reasons for this are unclear but subtle abnormalities in the dynamics of thyroid hormone secretion should be considered.

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Addendum

In order to assess the percentage of cases with fatigue in subjects with thyroid dysfunction that is due to the thyroid dysfunction, we calculated the attributable risk and population attributable risk. These calculations did not get through the review process of the manuscript. However, in the context of this thesis, the attributable risk provides clarity on the relationship between thyroid dysfunction and symptoms.

We calculated the attributable risk with use of the formula $(R_1\text{-}R_o)/R_1$, where R_1 is the risk of the exposed (prevalence of fatigue in subjects with thyroid dysfunction) and R_0 is the risk of the non-exposed (prevalence of fatigue in euthyroid subjects). The population attributable risk was calculated with the formula $(R\text{-}R_0)/R$, where R is the risk of fatigue in the total population and R_0 is the risk of the non-exposed (euthyroid subjects), in order to assess the percentage of cases with fatigue in the population that can be attributed to thyroid dysfunction (table 1).[1, 2]

The attributable risk was 14%, this means that only 14% of the cases of self-reported fatigue in the subjects with thyroid dysfunction could be attributed to thyroid dysfunction. The population attributable risk was 1%, thus only 1% of the cases of self-reported fatigue in the general population could be attributed to thyroid dysfunction.

Table 1.
A 2 x 2 table with disease frequency (thyroid dysfunction) and outcome (fatigue).

	Fatigue	**No fatigue**
Thyroid dysfunction	39.6% (R_1)	60.4%
Euthyroidism	34.0% (R_0)	66.0%

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CHAPTER 3

Association between thyroid function, thyroid autoimmunity and state and trait factors of depression.

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Abstract

Objective:

The aim of this study was to investigate whether thyroid function and thyroid peroxidase antibodies (TPOAb) are associated with depression, when using both state and trait parameters of depression.

Method:

In 1125 participants of the Nijmegen Biomedical Study, thyroid stimulating hormone (TSH), free thyroxine (FT_4) and TPOAb were measured twice. The Beck Depression Inventory (BDI), a self-reported lifetime diagnosis of depression and the neuroticism scale of the Eysenck Personality Questionnaire Revised Short Scale (EPQ-RSS) were used to evaluate the presence of state and trait features of depression.

Results:

We found no association between TSH and FT_4 levels and BDI score, current depression, lifetime diagnosis of depression and EPQ-RSS neuroticism score. Subjects with TPOAb had higher EPQ-RSS neuroticism scores in comparison to subjects without TPOAb, mean score 4.1 versus 3.2 (regression coefficient 0.70, 95% CI 0.1-1.3, p-value 0.02 after adjustment for confounders). The prevalence of a lifetime diagnosis of depression was higher in subjects with positive TPOAb in comparison to participants without TPOAb: 24.2% versus 16.7% (relative risk 1.4, 95% CI 1.0-2.1, p value 0.04 after adjustment for confounders).

Conclusion:

TPOAb are positively associated with trait markers of depression. The presence of TPOAb may be a vulnerability marker for depression.

Introduction

Thyroid dysfunction has been associated with mood disorders.[1-5] The general idea is that overt hypothyroidism can cause psychiatric diseases like depression, although in patients with depression, the incidence of overt thyroid dysfunction is low.[2, 4, 6, 7] Previous studies have shown a lack of relationship between subclinical or overt hypothyroidism and depression in the general population.[8-14]

A high-normal FT_4 and a low-normal TSH were associated with depression in some large population studies and a meta-analysis.[15-17] This finding is interesting. Within the normal range of thyroid function there is an association between serum TSH and FT_4 levels and several pathological conditions. Increased cardiovascular risk, blood pressure, serum cholesterol, body mass index and the presence of the metabolic syndrome are associated with a thyroid function within the low-normal range.[18-23] By contrast, atrial fibrillation is associated with a high-normal thyroid function.[24]

In the majority of cases, primary hypothyroidism is caused by autoimmune thyroiditis, and in these patients thyroid peroxidase autoantibodies (TPOAb) are frequently present. On the other hand, TPOAb are detected in 8-18% of the general population.[25] The association between the presence of TPOAb and depression remains unclear. A large population study found no association between the presence of TPOAb and depression, whereas other studies showed a positive association between the presence of TPOAb and depression in community based samples, cohorts of psychiatric patients and postmenopausal women. An association between the presence of TPOAb and a subsequent postpartum depression in pregnant women was also shown.[10, 26-31]

So far, most studies investigating the relationship between thyroid function and depression have only focused on state characteristics of depression, i.e. measures of the current presence of depressive symptoms. It may be questioned if thyroid function is more distinctly associated with trait markers, i.e. markers of long term vulnerability to depression such as lifetime diagnosis of depression and neuroticism, a personality trait which is a strong risk factor for depression.[32-35]

Aims of the study

The aim of this study was to investigate whether thyroid function, especially within the normal range, and the presence of thyroid peroxidase autoantibodies are associated with the prevalence and severity of depression in the general population, when using both state and trait markers of depression.

Subjects and Methods

Study participants

The subjects of this study are participants of the Nijmegen Biomedical Study (NBS), a large population-based survey performed in Nijmegen, a municipality in the eastern part of The Netherlands. Details of this study are described before.[25] Approval to conduct the study was obtained from the Institutional Review Board of the RUNMC. Of each age-group (range 5 years), 750 men and 750 women were invited to participate. Subjects of 85 years old or older were all invited to participate. A total of 22,451 age- and sex-stratified randomly selected adults received a questionnaire on lifestyle, medical history and symptoms in 2002-2003, of whom 9350 adults responded. Of 6343 subjects, blood samples were obtained and TSH and FT_4 were measured. The participants, who gave permission for further research, received a second questionnaire in 2005-2007. A total of 2253 respondents, in the age group 50 through 70 years, were invited to participate in a study of non-invasive measurements of atherosclerosis (NIMA) of whom 1517 gave their informed consent. In these subjects, blood samples for second TSH and FT_4 measurements and TPOAb measurements were taken between 2005 and 2008. Baseline and second TSH and FT_4 measurements were available for 1235 participants. We chose to include only the participants of whom two TSH measurements were available. When using only one measurement of TSH and FT_4, the intraindividual variation of TSH and FT_4 may result in an underestimation of the association between thyroid function and depression. For our analysis, we excluded subjects with known thyroid disease, those who used thyromimetic and/or thyrostatic drugs, and those who had former thyroid surgery and/or radioactive iodine treatment. In addition, we also excluded subjects using medication interfering with thyroid function such as lithium, amiodarone, kelp, oral glucocorticosteroids and/or dopamine agonists because of the possible effect of these medications on thyroid function. A total of 86 women and 24 men were excluded.

Laboratory measurements

Serum TSH was measured by an immunoluminometric assay on a random-access analyzer (Architect; Abbott Diagnostics Division). The reference interval used in our laboratory is 0.4-4.0 mIU/L. Serum FT_4 was measured with a luminescence enzyme immunoassay on a random-access assay system (Vitros ECI; Ortho Clinical Diagnostics). Our laboratory reference interval is 8.0-22.0 pmol/L. TPOAb were measured with a fluorescence immunoenzymometric assay for the quantitative measurement of the IgG class of anti-thyroperoxidase antibodies (AxSYM Anti-TPO; Abbott Diagnostics Division). The reference interval was defined as ≤12 kIU/L (data provided by manufacturer). More details about these measurements are described elsewhere.[25]

Questionnaire

The first and second questionnaire contained questions about gender, age, weight, height, lifestyle, symptoms, medical history including the presence of thyroid disease, depression and the use of medication. The presence of a lifetime diagnosis of depression was evaluated by the question: "Have you ever needed therapy for a depression, like medication, counseling or admission to a psychiatric hospital?" This question was included to detect the presence of a lifetime diagnosis of clinical relevant depression. The second questionnaire (filled out in 2005-2007) contained the Beck Depression Inventory IA (BDI) and the Eysenck Personality Questionnaire Revised Short Scale (EPQ-RSS).[36-40]

The BDI is a 21-question multiple-choice self-report inventory and one of the most widely used instruments for assessing symptoms of depression (alpha coefficient 0.86).[37] Participants rated the severity of each of the 21 symptoms on a 4-point scale ranging from 0 (symptom not at all present) to 3 (symptom severely present). A total score of 63 points is the maximum score. A score of 10 points was considered as a cut-off point for the presence of a depression.[36] When 1 or 2 items were missing, the scores of these items were imputed, using the average score of the other items of the BDI. When more than 2 items were missing, the total score was regarded as missing.

Neuroticism (range: 0–12) was measured using the Dutch version of the revised Eysenck Personality Questionnaire (EPQ-RSS). Results of the Dutch version of this questionnaire strongly resemble those of the English version (alpha coefficient 0.81-0.84).[41] The EPQ-RSS is based on a three-factor model of personality: neuroticism, extraversion and psychoticism. Neuroticism is a stable personality trait that also in later life can be measured reliably as it is not significantly affected by physical health variables.[42] When 1 or 2 items were missing, the scores of these items were imputed, using the average score of the other items of the neuroticism subscale of the EPQ-RSS. When more than 2 items were missing, the total score was regarded as missing. The maximum score on the neuroticism scale is 12 points.

The BDI score and the presence of depression were considered as state characteristics of depression, i.e. measures of the current presence of depressive symptoms. The lifetime diagnosis of depression and the EPQ-RSS neuroticism subscale score were studied as trait markers, i.e. markers of long-term vulnerability to depression.

Statistical analyses

For the analyses, we used the mean value of the baseline and second measurements of TSH and FT_4 in order to diminish the underestimation of the association between thyroid function and depression due to intraindividual variation of TSH and FT_4.

Because of a possible non-linear association between TSH, FT_4 and depression parameters and a skewed distribution of TSH we used tertiles of TSH and FT_4 within the normal range. Serum TSH and FT_4 levels were divided into 5 subclasses: serum level below the normal range, tertiles of serum level within the normal range and serum level above the normal range. Linear regression analysis was performed to calculate the regression coefficient (RC) and the 95% confidence interval (CI) of the association between the subclasses of TSH and FT_4, the presence of TPOAb and the BDI/EPQ-RSS neuroticism subscale score. Subjects with TSH or FT_4 within the middle tertiles were expected to have the lowest risk of depression and were defined as the reference group. To assess relative risks (RR) along with the 95% CI we used loglinear regression analyses (Poisson regression with robust standard errors), with the prevalence of current depression or lifetime diagnosis of depression as the dependent variable and thyroid function class or the presence of TPOAb as the independent variable.[43-45] We adjusted all analyses for age, gender and body mass index (BMI) because these factors had an influence on both depression and thyroid function. Further adjustment for smoking status and co-morbidity like cardiovascular disease, cancer, COPD, diabetes mellitus and renal disease did not change results and these variables were not included in the model. The chosen level of significance was 5%. We analysed the data with STATA version 11.0 (StataCorp, Texas).

Results

The characteristics of the 1125 subjects are shown in Table 1. Women had a higher BDI score, a higher EPQ-RSS neuroticism subscale score, a higher prevalence of TPOAb and a higher prevalence of a lifetime diagnosis of depression.

Table 1.
Characteristics of the study population.

Characteristic	Total N=1125		Male N=579		Female N=546	
Age (years)[a]	56.8	(± 5.7)	57.6	(± 5.7)	56.0	(± 5.6)
BMI (kg/m^2)[a]	25.6	(± 3.6)	26.0	(± 3.2)	25.2	(± 4.0)
TSH (mIU/L) '02-'03[1, b]	1.36	(0.98-1.95)	1.37	(0.99-1.91)	1.34	(0.96-2.01)
TSH (mIU/L) '05-'08[2, b]	1.31	(0.93-1.80)	1.28	(0.91-1.77)	1.34	(0.95-1.83)
FT_4 (pmol/L)'02-'03[1, b]	13.0	(11.8-14.2)	13.0	(11.7-14.2)	12.9	(11.8-14.2)
FT_4 (pmol/L) '05-'08[2, b]	13.4	(12.1-14.8)	13.3	(12.0-14.6)	13.5	(12.2-14.9)
TPOAb present (%)[c] N=1,124	135	(12.0%)	39	(6.7%)	96	(17.6%)
BDI score[c]	5.2	(± 4.7)	4.6	(± 4.6)	5.8	(± 4.8)
BDI score ≥10 (%) N=907	143	(15.8%)	64	(14.0%)	79	(17.6%)
EPQ-RSS neuroticism subscale score[c]	3.3	(± 2.8)	2.8	(± 2.6)	3.8	(± 2.8)
Lifetime diagnosis of depression (%)[c], N=1,007	177	(17.6%)	80	(15.7%)	97	(19.5%)

BMI, body mass index; TSH, thyroid stimulating hormone; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies; BDI, Beck Depression Inventory 1A; EPQ-RSS, Eysenck Personality Questionnaire Revised Short Scale.
[a] Mean ± standard deviation
[b] Median (interquartile range)
[c] In 1 subject TPOAb was missing, in 218 subjects the BDI score was missing, in 124 subjects the EPQ-RSS neuroticism subscale score was missing, in 118 subjects data on lifetime diagnosis of depression were missing.
[1] First measurement, 2002-2003
[2] Second measurement, 2005-2008

Table 2 shows the mean BDI scores by TSH and FT_4 subclasses and by presence of TPOAb. Within the normal range, subjects with TSH in the middle tertile and FT_4 in the lower tertile had the lowest BDI score (4.9 and 5.1, respectively). However, the differences in BDI scores were only small and there was no significant association found between BDI score and TSH and FT_4 subclasses, neither before nor after adjustment for gender, age and BMI, except for subjects with FT_4>22 pmol/l (regression coefficient (RC) 3.5, 95% CI 1.5-5.5, p-value <0.01). Subjects without TPOAb had lower BDI scores in comparison to subjects with TPOAb: 5.1 versus 6.0 respectively (RC 0.96, 95% CI 0.01 to 1.9, p-value 0.05). However, after adjustment for age, gender and BMI, there was no difference in BDI scores between these groups. Further analysis by gender did not change these results.

Table 2.
Mean Beck Depression Inventory 1A (BDI) score by TSH and FT_4 subclasses and by TPOAb after exclusion of subjects with known thyroid disease and the use of interfering medication.

		Total number[a]	BDI score	Difference from reference group (95% CI)[b]	
TSH[c]	<0.4 mIU/L	10	5.3	0.11	(-2.2 to 2.4)
	0.4-1.11 mIU/L	282	5.2	0.22	(-0.6 to 1.0)
	1.11-1.62 mIU/L[d]	298	4.9	-	-
	1.62-4.00 mIU/L	294	5.4	0.41	(-0.3 to 1.2)
	>4.00 mIU/L	23	5.9	0.82	(-1.4 to 3.0)
FT_4[c]	<8.0 pmol/L	2	3.0	-2.37	(-6.8 to 2.0)
	8-12.4 pmol/L	284	5.1	0.04	(-0.7 to 0.8)
	12.4-14.1 pmol/L[d]	297	5.2	-	-
	14.1-22 pmol/L	312	5.3	0.11	(-0.6 to 0.9)
	>22 pmol/L	3	8	3.48	(1.5 to 5.5)*
TPOAb	≤ 12 kIU/L[d]	791	5.1	-	-
	> 12 kIU/L	115	6.0	0.74	(-0.2 to 1.7)

BDI, Beck Depression Inventory 1A; TSH, thyroid stimulating hormone; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies; CI, confidence interval.

[a] Mean FT_4 was missing in 11 subjects, TPOAb was missing in 1 subject, the BDI score was missing in 218 subjects. These subjects included 2 subjects, with both mean FT_4 and the BDI score missing.

[b] Differences from reference group were obtained from linear regression analyses with adjustment for age, gender and body mass index.

[c] TSH and FT_4 are mean values of baseline and second measurements.

[d] Reference group

* p-value <0.05

Subjects with a BDI score of ≥10 were considered to have a current depression. Table 3 shows the prevalence of current depression by TSH and FT_4 subclasses and by TPOAb. Subjects with TSH and FT_4 in the middle tertile had the lowest prevalence of depression. However, there was no significant association between the subclasses of TSH and FT_4 or the presence of TPOAb and current depression, neither before nor after adjustment for age, gender and BMI. Again, further analysis by gender did not change these results.

Table 3.
Percentage of current depression by TSH and FT_4 subclasses and by TPOAb after exclusion of subjects with known thyroid disease and the use of interfering medication.

		Total number[a]	Current depression (%)	Relative Risk (95% CI)[b]	
TSH[c]	<0.4 mIU/L	10	20.0	1.5	(0.4 to 5.1)
	0.4-1.11 mIU/L	282	15.6	1.2	(0.8 to 1.8)
	1.11-1.62 mIU/L[d]	298	13.1	-	-
	1.62-4.00 mIU/L	294	17.7	1.3	(0.9 to 2.0)
	>4.00 mIU/L	23	26.1	2.0	(0.99 to 4.2)
FT_4[c]	<8.0 pmol/L	2	0	-	-
	8-12.4 pmol/L	284	16.6	1.1	(0.8 to 1.6)
	12.4-14.1 pmol/L[d]	297	15.5	-	-
	14.1-22 pmol/L	312	15.7	1.0	(0.7 to 1.5)
	>22 pmol/L	3	0	-	-
TPOAb	≤ 12 kIU/L[d]	791	15.3	-	-
	> 12kIU/L	115	19.1	1.2	(0.8 to 1.9)

TSH, thyroid stimulating hormone; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies; CI, confidence interval.
[a] Mean FT_4 was missing in 11 subjects, TPOAb was missing in 1 subject, the Beck Depression Inventory 1A (BDI) score was missing in 218 subjects. These subjects included 2 subjects, with both mean FT_4 and the BDI score missing.
[b] Relative risks were obtained from loglinear regression analyses with adjustment for age, gender and body mass index.
[c] TSH and FT_4 are mean values of baseline and second measurements.
[d] Reference group

Table 4 shows the mean EPQ-RSS neuroticism subscale scores by TSH and FT_4 subclasses and by the presence of TPOAb. Within the normal range, subjects in the middle tertile of TSH and FT_4 had lower EPQ-RSS neuroticism subscale scores in comparison to the other tertiles. However, the difference in EPQ-RSS neuroticism subscale score between the groups was only small and no significant association between the subclasses of TSH and FT_4 and the EPQ-RSS neuroticism subscale score was found, neither before nor after adjustment for gender, age and BMI.

Subjects with TPOAb had higher EPQ-RSS neuroticism subscale scores in comparison to subjects without TPOAb, 4.1 versus 3.2 respectively (RC 0.70, 95% CI 0.1 to 1.3, p-value 0.02 after adjustment for age, gender and BMI). Because women were more frequently positive for TPOAb and had higher EPQ-RSS neuroticism subscale scores, gender could be a major confounder. Therefore, analyses by gender were performed. Men with positive TPOAb had higher EPQ-RSS neuroticism subscale scores in comparison to men without TPOAb: 4.3 versus 2.7 respectively (RC 1.6, 95% CI 0.4 to 2.7, p-value <0.01 after adjustment for age and BMI). The results remained similar after exclusion of men with TSH or FT_4 outside the normal range. For women, no association between TPOAb and EPQ-RSS neuroticism subscale scores was found: women with positive TPOAb had only a slightly higher EPQ-RSS neuroticism subscale score in comparison to women without TPOAb: 4.0 versus 3.7, but this difference was not statistically significant, neither before nor after adjusment for confounders (RC 0.29, 95% CI -0.4 to 1.0, p-value 0.41 after adjustment for age and BMI).

Table 4.
Mean EPQ-RSS neuroticism subscale score by TSH and FT_4 subclasses and by TPOAb after exclusion of subjects with known thyroid disease and the use of interfering medication.

		Total number[a]	EPQ-RSS subscale score	Difference from reference group (95% CI)[b]	
TSH[c]	<0.4 mIU/L	11	3	-0.26	(-1.2 to 0.7)
	0.4-1.11 mIU/L	321	3.2	0.05	(-0.4 to 0.5)
	1.11-1.62 mIU/L[d]	328	3.1	-	-
	1.62-4.00 mIU/L	318	3.5	0.36	(-0.1 to 0.8)
	>4.00 mIU/L	23	4.0	0.80	(-0.4 to 2.0)
FT_4[c]	<8.0 pmol/L	3	4.3	1.30	(-1.2 to 3.8)
	8-12.4 pmol/L	317	3.3	0.15	(-0.3 to 0.6)
	12.4-14.1 pmol/L[d]	325	3.2	-	-
	14.1-22 pmol/L	341	3.3	0.05	(-0.4 to 0.5)
	>22 pmol/L	4	4.8	3.03	(-1.5 to 7.6)
TPOAb	≤ 12 kIU/L[d]	879	3.2	-	-
	> 12 kIU/L	121	4.1	0.70	(0.1 to 1.3)*

TSH, thyroid stimulating hormone; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies; EPQ-RSS, Eysenck Personality Questionnaire Revised Short Scale; CI, confidence interval.
[a] Mean FT_4 was missing in 11 subjects, TPOAb was missing in 1 subject, the EPQ-RSS neuroticism scale score was missing in 124 subjects.
[b] Differences from reference group were obtained from linear regression analyses with adjustment for age, gender and body mass index.
[c] TSH and FT_4 are mean values of baseline and second measurements.
[d] Reference group.
*p-value <0.05

Table 5 shows the prevalence of a lifetime diagnosis of depression by TSH and FT_4 subclasses and by presence of TPOAb. No association was found between the subclasses of TSH and FT_4 and lifetime diagnosis of depression, except for a higher prevalence of lifetime diagnosis of depression in subjects with FT_4 >22 pmol/L in comparison to subjects in the middle tertile of FT_4, 66.7% versus 19.0% (RR 4.6, 95% CI 1.9 to 11.3, p value <0.01). There was a positive association between the presence of TPOAb and the prevalence of a lifetime diagnosis of depression: the prevalence of lifetime diagnosis of depression was 24.2% in subjects with TPOAb versus 16.7% in subjects without TPOAb (RR 1.4, 95% CI 1.0 to 2.1, p value 0.04 after adjustment for age, gender and BMI). Further analyses by gender gave similar results for both men and women.

Table 5.
Percentage of lifetime diagnosis of depression by TSH and FT_4 subclasses and by TPOAb after exclusion of subjects with known thyroid disease and the use of interfering medication.

		Total number[a]	Lifetime depression (%)	Relative risk (95% CI)[b]	
TSH[c]	<0.4 mIU/L	11	9.1	0.6	(0.1 to 3.8)
	0.4-1.11 mIU/L	323	16.4	1.0	(0.7 to 1.4)
	1.11-1.62 mIU/L[d]	329	16.4	-	-
	1.62-4.00 mIU/L	320	20.0	1.2	(0.8 to 1.6)
	>4.00 mIU/L	24	20.8	1.3	(0.6 to 3.0)
FT_4[c]	<8.0 pmol/L	3	33.3	1.8	(0.5 to 6.2)
	8-12.4 pmol/L	320	18.1	1.0	(0.7 to 1.4)
	12.4-14.1 pmol/L[d]	327	19.0	-	-
	14.1-22 pmol/L	343	15.5	0.9	(0.6 to 1.2)
	>22 pmol/L	3	66.7	4.6	(1.9 to 11.3)*
TPOAb[c]	≤ 12 kIU/L[d]	882	16.7	-	-
	> 12kIU/L	124	24.2	1.4	(1.0 to 2.1)*

TSH, thyroid stimulating hormone; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies; CI, confidence interval.
[a] Mean FT_4 was missing in 11 subjects, TPOAb was missing in 1 subject, data on lifetime diagnosis of depression were missing in 118 subjects.
[b] Relative risks were obtained from loglinear regression analyses with adjustment for age, gender and body mass index.
[c] TSH and FT_4 are mean values of baseline and second measurements.
[d] Reference Group
* p-value <0.05

Discussion

In our population-based study, we found no association between thyroid function and state or trait factors of depression. There were only few subjects with a mean value of TSH or FT_4 outside the normal range, so analyses of those subclasses of TSH and FT_4 should be interpreted with caution.

Our results are in line with a number of previous studies, including a large population study with 30,000 participants, performed in Norway in 1995 (the HUNT-study), which also found no association between thyroid function classes and depression after adjustment for gender and age.[8-12] In contrast to these studies, several other studies suggested that a FT_4 level in the upper-normal range and a TSH level in the low-normal range are associated with an increased risk of depression.[15-17] We could not confirm these results. It is difficult to compare these studies because of their different study designs. In the various studies, the composition of the population differed considerably. Some studies included only male subjects or young adults, whereas our study had a restrictive age range of 50 to 70 years. Some studies used a clinical sample instead of a population based sample. Also, the studies used different clinical tests or no validated test at all to assess the presence of depression.

Because the course of symptoms of depression has been shown to be variable, we hypothesized that trait markers for depression, like the personality trait neuroticism and a lifetime diagnosis of depression, might be more distinctly associated with thyroid function than state factors. In our study, we found no association between thyroid function and these trait markers. There are only few studies that have studied the relationship between thyroid function and trait markers instead of state markers of depression and these studies yielded conflicting results. In accordance with our results, Forman et al. found no association between lifetime diagnosis of depression and thyroid function.[15] Frey et al found that low TSH levels in 121 healthy subjects were associated with neuroticism.[46] Perhaps the differences in results can be explained by the differences in study populations. For example, Frey et al. recruited mostly young volunteers in comparison to the population-based sample, aged between 50 and 70 years, in the present study. Also, Frey et al. used a different kind of instrument to evaluate neuroticism (NEO Five-Factor Inventory).

Some previous studies have reported a higher prevalence of TPOAb in patients with mood disorders or a positive association between the presence of TPOAb and depression.[10, 28, 29, 31] These studies comprised small sample sizes of selected groups of subjects, such as pregnant women, perimenopausal women and psychiatric patients. We did not find a significant

relationship between the presence of TPOAb and state markers of depression in the general population. Our results are concordant with another large population based study, which showed no relationship between the presence of TPOAb and state markers of depression.[27]

However, we did find an association between the presence of TPOAb and trait factors like neuroticism and lifetime diagnosis of depression. To our knowledge, this is the first study examining the relationship between the presence of TPOAb and neuroticism. Carta et al. also found an association between the presence of a lifetime diagnosis of mood or anxiety disorder and TPOAb in both men and women.[26] A history of bipolar disorder can also be regarded as a trait characteristic, because it represents lifetime vulnerability. Previous studies have reported a higher prevalence of positive TPOAb in patients with a bipolar disorder.[47] Vonk et al. concluded from a twin study that autoimmune thyroiditis is not only related to bipolar disorder itself but also to the genetic vulnerability to develop this disorder, whereas Hillegers et al. found bipolar offspring to be more vulnerable to develop thyroid autoimmunity independently from the vulnerability to develop psychiatric disorders.[48, 49] This study showed that the presence of TPOAb is associated with trait characteristics of depression, whereas thyroid function is not. This finding suggests that the higher prevalence of current thyroid dysfunction in subjects with TPOAb is not the cause of the association between TPOAb and trait characteristics of depression.[25] However, as neuroticism and life time depression are trait characteristics, an association between thyroid dysfunction and depression may have been present in the past. Alternatively, we hypothesize that the association between TPOAb and trait characteristics of depression does not reflect a causal relationship but more likely is the result of a co-association with another factor, like predisposition for auto-immunity.

The strength of our study is that we measured thyroid function twice. Most of the reported associations between thyroid function and depression in population-based studies are based on one single measurement of TSH and/or FT_4. These associations might be underestimated due to the intraindividual variation of the TSH and FT_4 measurements. Other strengths of our study are the fact that we measured both trait and state characteristics of depression and the large sample size.

Our study has some limitations. As already mentioned, there were only few subjects with a mean value of TSH or FT_4 outside the normal range, so analyses of those subclasses of TSH and FT_4 should be interpreted cautiously. Second, the BDI has been validated as an excellent screening instrument for the presence of depression.[36-38] However, budget constraints did not permit us to perform a clinical evaluation to diagnose a depression. Third, due to the nature of the NIMA study, our study population comprised only subjects between 50 and 70 years of age.

Further studies will have to confirm our results in other age groups. Finally, we cannot rule out selection bias due to a difference in depression or thyroid function among the responders versus non-responders.

In conclusion, we found no association between thyroid function and the state and trait markers of depression. The presence of TPOAb was not associated with state markers but had a positive association with trait markers of depression. We therefore conclude that the presence of TPOAb may be a vulnerability marker for depression.

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CHAPTER 4

Associations between thyroid function and mortality: the influence of age.

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Abstract

Objective

The aim of this study was to investigate the influence of age on the association between thyroid function and mortality.

Design

The Nijmegen Biomedical Study is a population-based study comprising 5816 randomly selected adults of all age groups without previously known thyroid disease.

Methods

Thyroid stimulating hormone (TSH), free thyroxine (FT_4) and peroxidase antibodies were measured in 2002-2003. The number of deaths were established in 2012 (median follow up time 9.4 years).

Results

Subclinical thyrotoxicosis was associated with mortality in subjects < 65 years old (HR 2.5, 95% CI 1.1-5.7), but not in subjects > 65 years old. As for thyroid function within the normal range: in the 493 participants aged 80 years or older, a FT_4 level in the high-normal range (18.5-22 pmol/l) was associated with a higher mortality in comparison to FT_4 levels in the middle range (11.5-15.0 pmol/L): HR 1.7 (95% CI 1.0-2.9). In these elderly, also TSH levels within the high-normal range (3.0-4.0 mIU/L) were associated with a higher mortality in comparison to TSH levels within the middle range (1.0-2.0 mIU/L): HR 1.8 (95% CI 1.0-3.1).

Conclusions

The relationship between thyroid function and mortality differs according to age. This finding might (partially) explain the discrepant results of previous studies examining the relationship between thyroid function and mortality in different age groups.

Introduction

Thyroid function disorders are common in the general population. The prevalence varies among different populations, depending on the iodine intake of the population. Overt thyroid dysfunction is associated with several cardiovascular risk factors, morbidity and mortality.[1, 2] Subclinical thyroid function disorders are also associated with cardiovascular risk factors and cardiovascular diseases, although the associations are generally less strong and more controversial due to conflicting results in numerous studies.[3, 4] Whether subclinical thyroid dysfunction is associated with mortality remains controversial with some studies finding an association between mortality and subclinical hypothyroidism[5-10] and/or subclinical hyperthyroidism,[7] while other studies do not confirm these results.[11-15] Recently 6 meta-analyses reported conflicting results. These studies had to deal with clinical heterogeneity among the studies due to differences in both the included populations and methods of adjustment for confounders.[16-21]

In the past few years, the effects of thyroid function within the reference range on various biological parameters have been studied.[22] Only few studies investigated the relationship between thyroid function within the normal range and mortality. These studies also reported conflicting results.[23-29] Some found a positive association between mortality and blood TSH concentration within the normal range,[23] whereas others found a negative[24, 28] or no association.[25-27, 29] In addition, a positive association between mortality and FT_4 within the normal range has been reported in elderly.[24, 27, 29]

These latter studies suggest that age might influence the association between thyroid function and mortality. However, the total number of young participants and oldest elderly is limited in most studies. Some studies, including 2 meta-analyses, found an association between subclinical thyroid dysfunction and mortality in younger, but not in older subjects.[9, 17, 18] Furthermore, the results of the Leiden 85+ Study suggest a protective effect of higher TSH levels in the oldest elderly.[24] Other studies could not confirm this effect, but they did show a beneficial effect of lower FT_4 levels, even within the normal range, in the elderly.[27, 29] Most studies have been performed in iodine sufficient populations, in which TSH increases with age. In populations with a (mild) iodine insufficiency at present or in the past, TSH decreases with age and FT_4 increases with age.[30,32]

The aim of this study was to investigate the influence of age on the association between thyroid function and mortality, in a population in which TSH decreases and FT_4 increases with age.

Materials and Methods

Study participants

The subjects of this study are participants of the Nijmegen Biomedical Study (NBS), a large population-based survey performed in 2002-2003 in Nijmegen, a municipality in the eastern part of The Netherlands. Details of this study have been described before.[30] In the past, mild iodine deficiency was present in this part of the Netherlands.[33, 34] Currently, the iodine status of this population is considered to be adequate.[35, 36] Approval to conduct the study was obtained from the Institutional Review Board. A total of 22,451 age- and sex-stratified randomly selected adults received a questionnaire on gender, age, weight, height, lifestyle, medical history and the use of medication. Of each 5-year age-group, 750 men and 750 women were invited to participate. We excluded the subjects with known thyroid disease (overt/subclinical thyrotoxicosis or hypothyroidism), those who used thyromimetic and/or thyrostatic drugs, and those who had former thyroid surgery and/or radioactive iodine treatment. Also, participants who were pregnant or used medication interfering with thyroid function such as lithium, amiodarone, oral glucocorticosteroids, kelp, dopamine agonists and/or opiates were excluded because of the possible influence of pregnancy and these medications on serum TSH and FT_4 levels.

Data on vital status and changes in address were obtained from the municipal registers at set times. For respondents who died, the date of death was traced through death certificates from municipal registers until October, 2012. When subjects moved out of the region and no data on vital status could be obtained anymore, data were used for analyses until the date of moving out (interval censuring).

Laboratory methods

Blood samples were taken in 2002-2003 in order to measure thyroid stimulating hormone (TSH), free thyroxine (FT_4) and peroxidase antibodies. Serum TSH was measured by an immunoluminometric assay on a random-access analyzer (Architect; Abbott Diagnostics Division). The reference interval used in our laboratory is 0.4-4.0 mIU/L. Serum FT_4 was measured with a luminescence enzyme immunoassay on a random-access assay system (Vitros ECI; Ortho Clinical Diagnostics). Our laboratory reference interval is 8.0-22.0 pmol/L. TPOAbs were measured with a fluorescence immunoenzymometric assay for the quantitative measurement of the IgG class of anti-thyroperoxidase antibodies (AxSYM Anti-TPO; Abbott Diagnostics Division). The reference interval was defined as <12 kIU/L (data provided by manufacturer). Thyroid function was classified as overt thyrotoxicosis if TSH was <0.4 mIU/L and FT_4 was >22 pmol/L and it was classified as subclinical thyrotoxicosis if TSH was <0.4 mIU/L and

FT_4 was ≥8 pmol/L and ≤ 22 pmol/L. Thyroid function was classified as overt hypothyroidism if TSH was >4.0 mIU/L and FT_4 was <8 pmol/L and as subclinical hypothyroidism if TSH was >4.0 mIU/L and FT_4 was ≥8 pmol/L and ≤ 22 pmol/L. When both TSH and FT_4 were within the normal range, thyroid function was classified as euthyroidism. When either TSH or FT_4 was not within the normal range, thyroid function was classified as thyroid dysfunction.

Statistical analyses

We produced Kaplan-Meier curves to compare unadjusted survival ratios of participants according to the thyroid function class and according to subclasses of TSH and FT_4 within the normal range. For this purpose, we stratified the euthyroid participants according to the TSH level (TSH 0.4-1.0, 1.0-2.0, 2.0-3.0, 3.0-4.0 mIU/L) and FT_4 level (8.0-11.5, 11.5-15.0, 15.0-18.5, 18.5-22.0 pmol/L).

Using a Cox proportional hazards model, we calculated adjusted hazard ratios (HR) with a 95% confidence interval (CI) of mortality by thyroid function classes, using the euthyroid group as the reference group. In addition, we calculated adjusted hazard ratios (HR) with a 95% confidence interval (CI) of mortality of the TSH and FT_4 subclasses within the normal range in euthyroid subjects. We adjusted all analyses for possible confounders, selecting the following factors for adjustment because of their known or presumed relation with both thyroid function and mortality: age, gender, body mass index (BMI), smoking status, medical history of hypertension, hypercholesterolemia, diabetes mellitus, cardiovascular disease, cancer, deep venous thrombosis, asthma/COPD, rheumatoid arthritis, renal and/or liver disease. Log minus log plots and inclusion of time dependent covariates in the Cox model were used to check the proportional hazards model assumption, which was not violated in any of the models. To assess the influence of age on the relationship between mortality and thyroid function, we subdivided the participants into three age groups: age < 65 years, age 65-80 years and age ≥ 80 years old and performed the analyses as described above in each subgroup. All statistical analyses were performed using STATA version 11 (StataCorp, Texas).

Results

A total of 9350 subjects responded to the invitation to fill out the questionnaire (response rate: 42%). Of these responders, 6434 (69%) subjects gave permission for blood withdrawal. The demographic characteristics of the non-responders differed only slightly from the responders, who gave permission for blood withdrawal: the mean age was 53.1 versus 56.1 years respectively, the percentage of women was 50.2% versus 53.8%. We excluded 47 pregnant women and 212 subjects using medication interfering with thyroid function. In addition, we excluded 322 subjects because of previously known thyroid disease. Of 37 subjects, the date of blood collection was missing or the follow up was completely missing and they were also excluded. The median follow up time was 9.4 years. Of the remaining 5816 participants, 775 subjects (13.3%) died within the follow up period of the study. Table 1 shows the population characteristics. Data on BMI were missing in 137 subjects, data on smoking status were missing in 17 subjects and data on medical history were missing in 54 subjects.

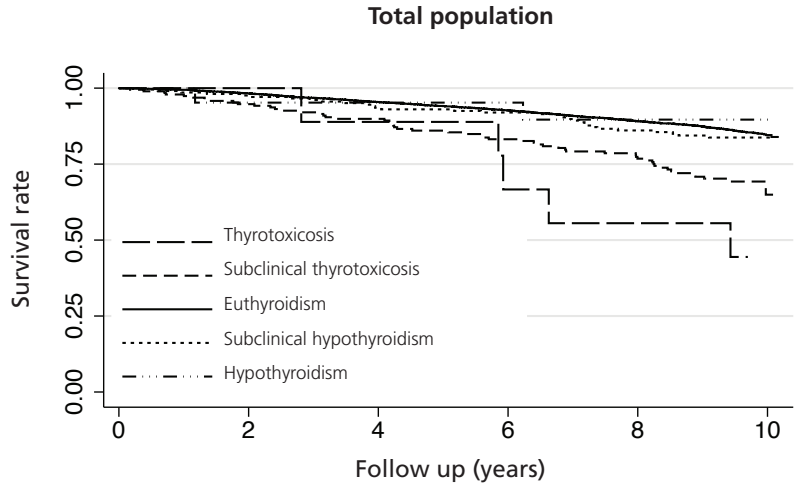
The Kaplan-Meier survival curves for different thyroid categories are depicted in figure 1. The lowest survival rates were observed in subjects with thyrotoxicosis. Subclinical thyrotoxicosis was also associated with lower survival rates, especially in subjects < 65 years old. The Kaplan-Meier survival curves for TSH and FT_4 subclasses in euthyroid subjects are shown in figure 2 and 3. Subjects with a high-normal FT_4 level (18.5-22.0 pmol/L) had the lowest survival rates in subjects ≥ 65 years old but no difference in survival rates between FT_4 subclasses was found in subjects <65 years old.

Table 1.
Characteristics of the population.

Characteristic	Total N=5816		Age < 65 years N=3773		Age 65-80 years N=1550		Age ≥ 80 years N=493	
Number of deaths	775	(13.3%)	110	(2.9%)	362	(23.4%)	303	(61.5%)
Age (years)[a]	55.7	± 17.9	45.3	± 12.9	71.9	±4.3	84.0	±3.1
Male	2761	(47.5%)	1611	(42.7%)	875	(56.5%)	275	(55.8%)
BMI (kg/m^2)[a]	25.1	± 4.0	24.8	± 4.2	25.9	± 3.7	25.2	±3.8
Smoking no. (%)	1301	(22.4%)	1004	(26.7%)	241	(15.6%)	56	(11.4%)
Medical history of:								
- COPD/asthma (%)	712	(12.4%)	443	(11.8%)	203	(13.3%)	66	(13.6%)
- CVD (%)	577	(10.0%)	133	(3.6%)	312	(20.4%)	132	(27.1%)
- rheumatic disease (%)	482	(8.4%)	192	(5.1%)	220	(14.4%)	70	(14.4%)
- cancer (%)	446	(7.7%)	162	(4.3%)	200	(13.1%)	84	(17.3%)
- diabetes mellitus (%)	315	(5.5%)	110	(2.9%)	154	(10.1%)	51	(10.5%)
- hypertension (%)	1365	(25.5%)	649	(18.0%)	547	(40.9%)	169	(41.5%)
- hypercholesterolemia (%)	899	(17.1%)	407	(11.4%)	429	(33.0%)	63	(17.0%)
- VTE (%)	221	(3.8%)	66	(1.8%)	99	(6.5%)	56	(11.5%)
- kidney disease (%)	171	(3.0%)	85	(2.3%)	51	(3.3%)	35	(7.1%)
- liver disease (%)	134	(2.3%)	89	(2.4%)	35	(2.3%)	10	(2.1%)
TPOAbs positive	735	(12.6%)	448	(11.9%)	234	(15.1%)	53	(10.8%)
TSH (mIU/L)[b]	1.34	(0.34-4.92)	1.41	(0.43-4.72)	1.24	(0.26-5.58)	1.15	(0.21-5.68)
FT_4 (pmol/L)[b]	13.3	(9.7-18.2)	13.0	(9.4-17.4)	13.6	(9.9-19.0)	14.3	(10.3-19.3)

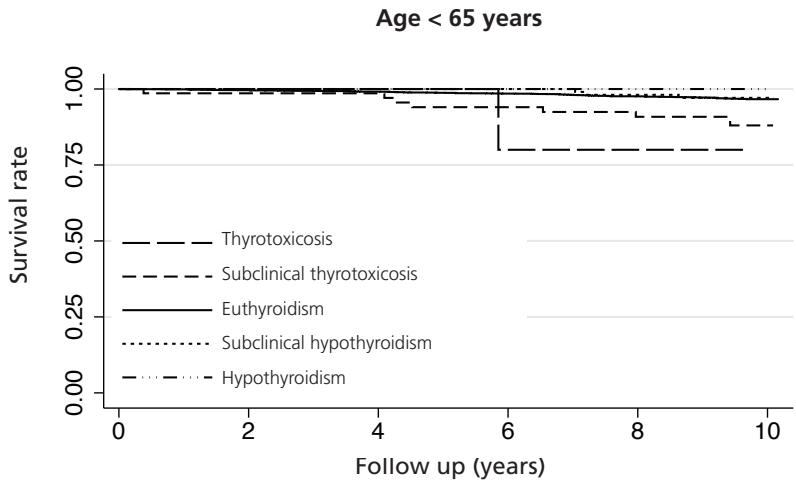
BMI, body mass index; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; VTE, venous thromboembolism; TPOAbs, peroxidase antibodies; TSH, thyroid stimulating hormone; FT_4, free thyroxine.
[a] Plus-minus values are means ± standard deviations;
[b] Geometric mean, 2.5th-97.5th percentiles.

Figure 1
Kaplan-Meier survival curves by thyroid function class, stratified for age.



Number at risk

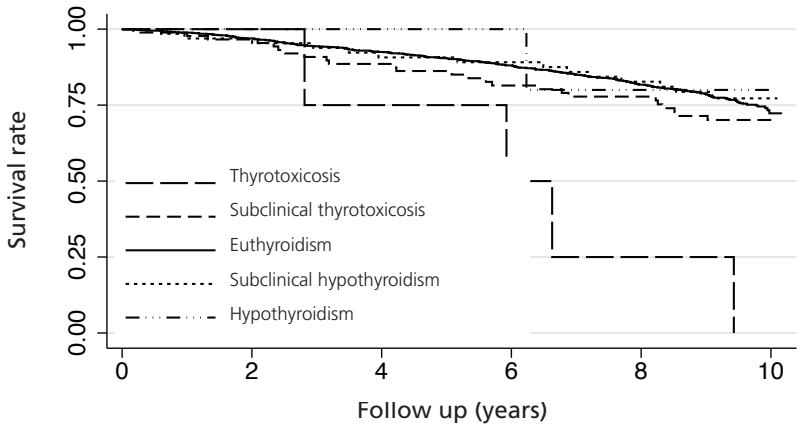
Thyrotoxicosis	9	9	8	6	5
Subcl. thyrotoxicosis	193	176	164	146	129
Euthyroidism	5365	5084	4693	4347	4044
Subcl. hypothyroidism	212	197	183	174	157
Hypothyroidism	22	20	20	17	15



Number at risk

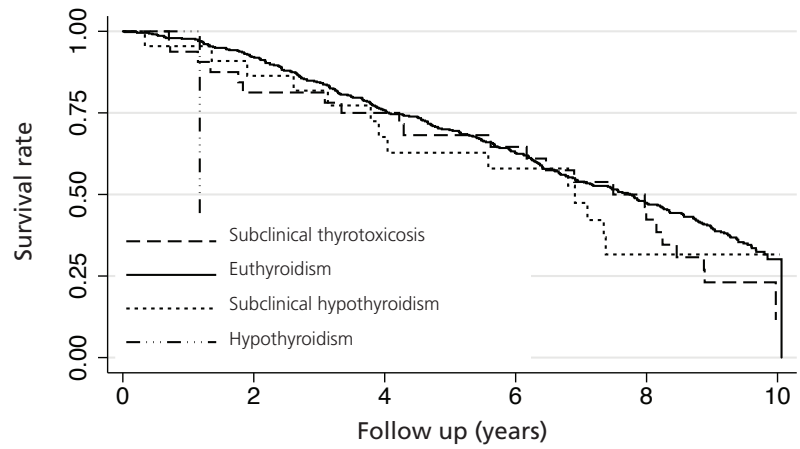
Thyrotoxicosis	5	5	5	4	4
Subcl. thyrotoxicosis	72	66	66	60	57
Euthyroidism	3547	3365	3145	2963	2846
Subcl. hypothyroidism	125	115	111	106	101
Hypothyroidism	15	15	15	12	11

Age 65-80 years



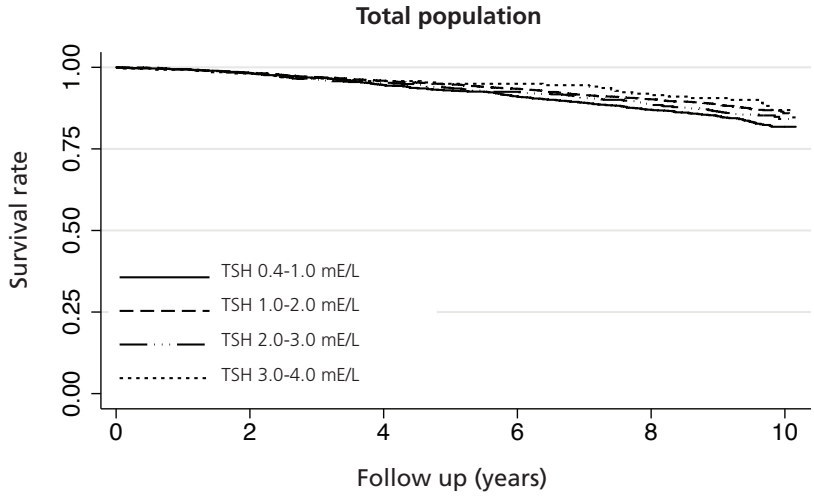
Number at risk					
Thyrotoxicosis	4	4	3	2	1
Subcl. thyrotoxicosis	89	84	76	68	61
Euthyroidism	1381	1322	1233	1137	1030
Subcl. hypothyroidism	65	63	58	56	50
Hypothyroidism	6	5	5	5	4

Age ≥ 80 years

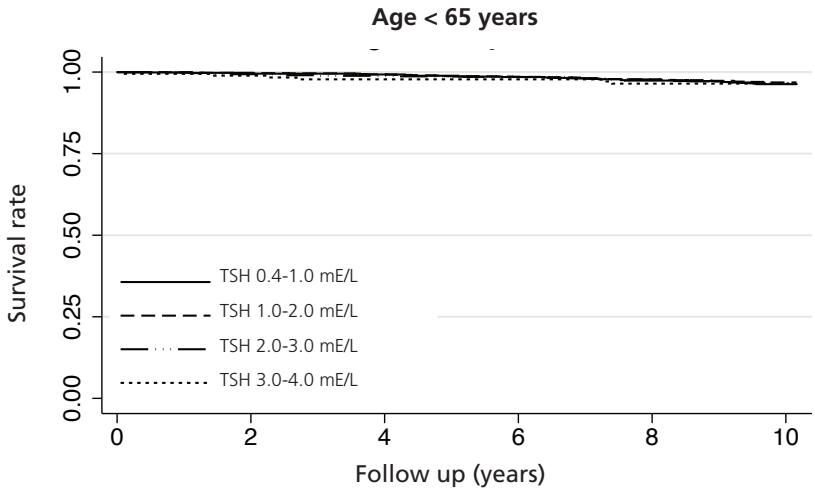


Number at risk					
Subcl. thyrotoxicosis	32	26	22	18	11
Euthyroidism	437	397	315	247	168
Subcl. hypothyroidism	22	19	14	12	6
Hypothyroidism	1	0	0	0	0

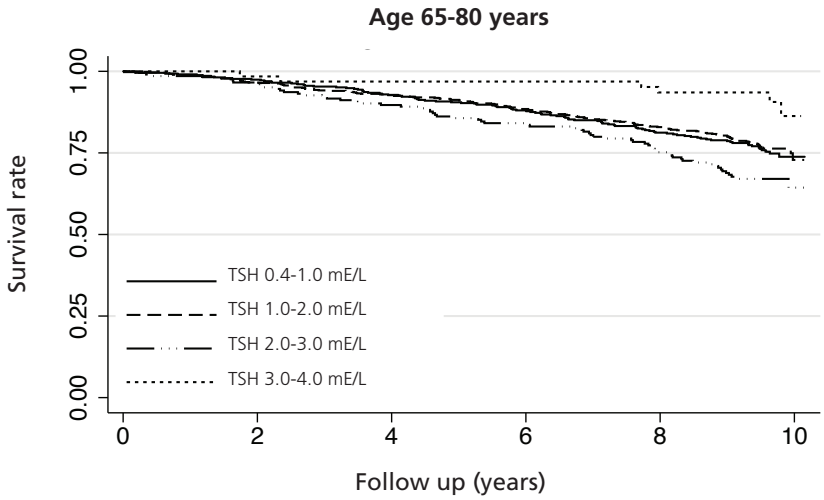
Figure 2
Kaplan-Meier survival curves by TSH subclass in euthyroid subjects, stratified for age.



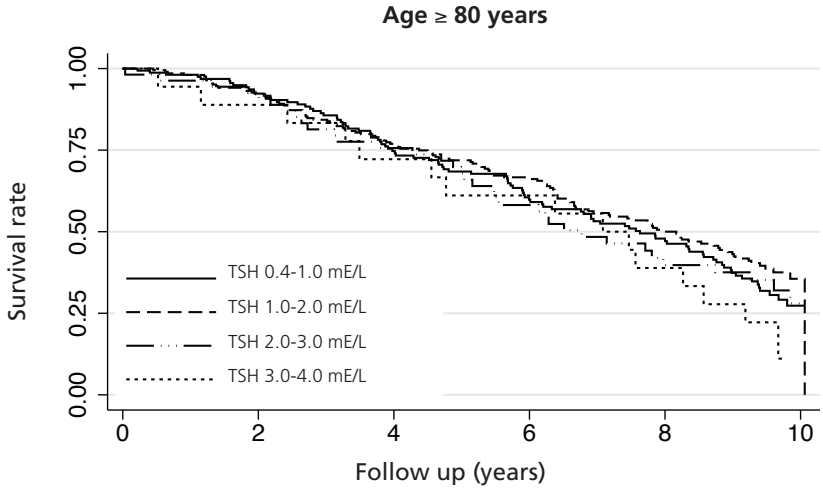
Number at risk						
TSH 0.4-1.0 mE/L	1468	1382	1263	1163	1080	84
TSH 1.0-2.0 mE/L	2719	2598	2410	2239	2087	163
TSH 2.0-3.0 mE/L	906	851	781	719	672	70
TSH 3.0-4.0 mE/L	272	253	239	226	205	28



Number at risk						
TSH 0.4-1.0 mE/L	873	821	771	729	703	64
TSH 1.0-2.0 mE/L	1840	1765	1647	1552	1490	125
TSH 2.0-3.0 mE/L	646	605	562	526	511	60
TSH 3.0-4.0 mE/L	188	174	165	156	142	24

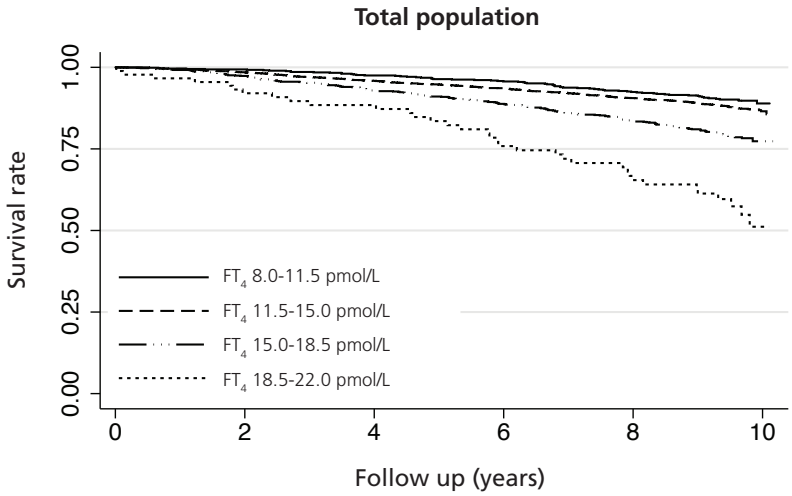


Number at risk						
TSH 0.4-1.0 mE/L	434	418	384	351	317	19
TSH 1.0-2.0 mE/L	675	645	608	564	514	32
TSH 2.0-3.0 mE/L	206	196	180	163	143	9
TSH 3.0-4.0 mE/L	66	63	61	59	56	4



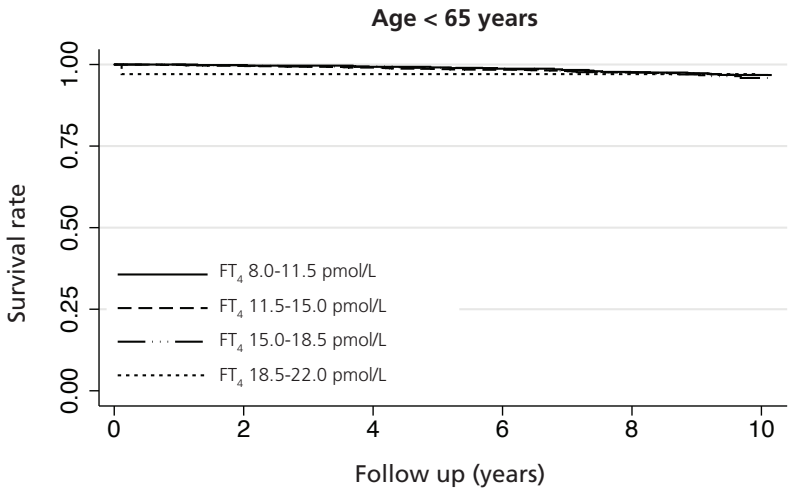
Number at risk						
TSH 0.4-1.0 mE/L	161	143	108	83	60	1
TSH 1.0-2.0 mE/L	204	188	155	123	83	6
TSH 2.0-3.0 mE/L	54	50	39	30	18	1
TSH 3.0-4.0 mE/L	18	16	13	11	7	0

Figure 3
Kaplan-Meier survival curves by FT_4 subclass in euthyroid subjects, stratified for age.



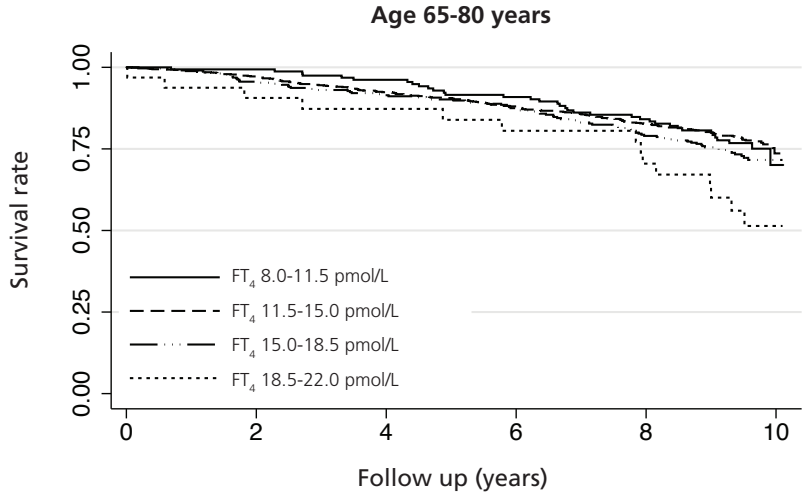
Number at risk

FT_4 8.0-11.5 pmol/L	798	767	712	660	617	49
FT_4 11.5-15.0 pmol/L	3472	3293	3050	2846	2666	229
FT_4 15.0-18.5 pmol/L	1006	944	858	782	711	64
FT_4 18.5-22.0 pmol/L	89	80	73	59	50	3



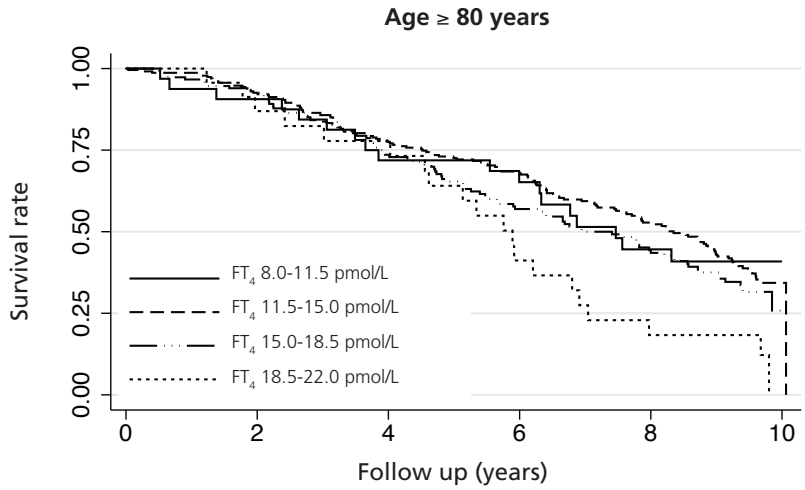
Number at risk

FT_4 8.0-11.5 pmol/L	606	582	542	505	482	43
FT_4 11.5-15.0 pmol/L	2370	2247	2100	1984	1910	186
FT_4 15.0-18.5 pmol/L	537	505	473	448	429	44
FT_4 18.5-22.0 pmol/L	34	31	30	26	25	0



Number at risk

FT_4 8.0-11.5 pmol/L	160	156	147	136	123	6
FT_4 11.5-15.0 pmol/L	870	834	776	717	657	39
FT_4 15.0-18.5 pmol/L	319	303	284	260	229	16
FT_4 18.5-22.0 pmol/L	32	29	26	24	21	3



Number at risk

FT_4 8.0-11.5 pmol/L	32	29	23	19	12	0
FT_4 11.5-15.0 pmol/L	232	212	174	145	99	4
FT_4 15.0-18.5 pmol/L	150	136	101	74	53	4
FT_4 18.5-22.0 pmol/L	23	20	17	9	4	0

Table 2 shows the number of deaths within the follow up period and the hazard ratios by thyroid function class, stratified for age and adjusted for possible confounders. Seven subjects had a serum TSH >0.4 mIU/L and FT_4 >22 pmol/L, 8 subjects had a serum TSH value of <0.4 mIU/L and FT_4 <8 pmol/L and were not assigned to one of the thyroid function classes. They were assigned to the thyroid dysfunction group. Thyroid dysfunction (either TSH or FT_4 not within the normal range) was associated with a higher mortality in comparison to euthyroidism, 21.3 versus 12.7%, HR 1.3 (95% CI 1.0-1.6) after adjustment for possible confounders. The difference was more distinct in subjects < 65 years old: 5.3% versus 2.8%, HR 1.7 (95% CI 0.9-3.2). The results of subjects with overt hypothyroidism and thyrotoxicosis should be interpreted cautiously, as only a limited number of participants were found to have an overt thyroid dysfunction. Mortality was higher in subjects with overt thyrotoxicosis in comparison to subjects with euthyroidism, 55.6% versus 12.7%, HR 8.2 (95% CI 3.4-19.7). Subclinical thyrotoxicosis was only associated with mortality in subjects < 65 years old, 9.7% versus 2.8% (HR 2.5 (95% CI 1.1-5.7), but not in subjects aged 65 years or older.

Table 2.
Number of deaths and the hazard ratios by thyroid function classification.

	Total population		< 65 years		65-80 years		≥ 80 years	
	Deaths % (n)	**HR (95% CI)**	**Deaths % (n)**	**HR (95% CI)**	**Deaths % (n)**	**HR (95% CI)**	**Deaths % (n)**	**HR (95% CI)**
Euthyroidism	12.7% 679/5365	Reference	2.8% 98/3547	Reference	23.0% 317/1381	Reference	60.4% 264/437	Reference
Thyroid dysfunction	21.3% 96/451	1.3 (1.0-1.6)	5.3% 12/226	1.7 (0.9-3.2)	26.6% 45/169	1.3 (0.9-1.7)	69.6% 39/56	1.3 (0.9-1.8)
- Overt thyrotoxicosis	55.6% 5/9	8.2 (3.4-19.7)	20.0% 1/5	13.4 (1.8-98.1)	100% 4/4	7.8 (2.9-21.2)	0.0% 0/0	No subjects
- Subclinical thyrotoxicosis	28.5% 55/193	1.2 (0.9-1.6)	9.7% 7/72	2.5 (1.1-5.7)	28.1% 25/89	1.3 (0.8-2.0)	71.9% 23/32	1.1 (0.7-1.8)
- Subclinical hypothyroidism	14.6% 31/212	1.2 (0.8-1.7)	2.4% 3/125	0.9 (0.3-3.0)	21.5% 14/65	1.0 (0.6-1.8)	63.6% 14/22	1.5 (0.9-2.5)
- Overt hypothyroidism	9.1% 2/22	1.6 (0.4-6.6)	0.0% 0/15	No deaths	16.7% 1/6	1.6 (0.2-11.7)	100% 1/1	35.1 (4-268)

Cox regression adjusted for age, gender, BMI, smoking status, medical history of hypertension, hypercholesterolemia, diabetes mellitus, cardiovascular disease, cancer, deep venous thrombosis, asthma/COPD, rheumatoid arthritis, renal and/or liver disease.

Table 3 shows the number of deaths and the hazard ratios in euthyroid subjects by TSH and FT_4 subclasses within the normal range. A FT_4 level in the high-normal range (18.5-22 pmol/l) was associated with a higher mortality in comparison to FT_4 levels in the middle range (11.5-15.0 pmol/L): 39.3% versus 11.0%, HR 1.6 (95% CI 1.1-2.4). Sub analysis by age showed that this association was only present in subjects ≥ 80 years old (HR 1.7 (95% CI 1.0-2.8)). TSH levels within the high-normal range (TSH 3.0-4.0 mIU/L) were associated with a higher mortality in comparison to TSH levels in the middle range (1.0-2.0 mIU/L) in subjects >80 years old: 83.3 % versus 57.4 %, HR 1.8 (95% CI 1.0-3.1) which was not the case in subjects <80 years old.

There was no association between the presence of TPOAbs and mortality in the total population (HR 0.9, 95% CI 0.7-1.2) nor in the different age groups (data not shown).

Table 3.
Number of deaths and the hazard ratios in euthyroid subjects by TSH and FT_4 subclasses within the normal range.

	Total population		< 65 years		65-80 years		≥ 80 years	
	Deaths % (n)	**HR (95% CI)**	**Deaths % (n)**	**HR (95% CI)**	**Deaths % (n)**	**HR (95% CI)**	**Deaths % (n)**	**HR (95% CI)**
TSH 0.4-1.0 mIU/L	15.1% 221/1468	1.1 (0.9-1.3)	3.0% 26/873	0.9 (0.5-1.4)	22.6% 98/434	1.1 (0.9-1.4)	60.3% 97/161	1.1 (0.9-1.5)
TSH 1.0-2.0 mIU/L	11.5% 313/2719	Reference	2.6% 48/1840	Reference	21.9% 148/675	Reference	57.4% 117/204	Reference
TSH 2.0-3.0 mIU/L	13.0% 118/906	1.3 (1.0-1.6)	2.8% 18/646	1.2 (0.7-2.2)	31.6% 65/206	1.5 (1.1-2.0)	64.8% 35/54	1.1 (0.7-1.6)
TSH 3.0-4.0 mIU/L	9.9% 27/272	0.9 (0.6-1.3)	3.2% 6/188	1.4 (0.6-3.3)	9.1% 6/66	0.2 (0.1-0.7)	83.3% 15/18	1.8 (1.0-3.1)
FT_4 8.0-11.5 pmol/L	8.8% 70/798	1.1 (0.8-1.4)	2.6% 16/606	1.0 (0.6-1.7)	22.5% 36/160	1.1 (0.8-1.6)	56.3% 18/32	1.0 (0.6-1.8)
FT_4 11.5-15 pmol/L	11.0% 383/3472	Reference	2.7% 64/2370	Reference	21.3% 185/870	Reference	57.8% 134/232	Reference
FT_4 15-18.5 pmol/L	19.0% 191/1006	1.1 (0.9-1.3)	3.2% 17/537	0.8 (0.4-1.3)	25.7% 82/319	1.2 (0.9-1.5)	61.3% 92/150	1.1 (0.8-1.5)
FT_4 18.5-22 pmol/L	39.3% 35/89	1.6 (1.1-2.4)	2.9% 1/34	1.0 (0.1-7.2)	43.8% 14/32	1.6 (0.9-3.1)	87.0% 20/23	1.7 (1.0-2.9)

Cox regression adjusted for age, gender, BMI, smoking status, medical history of hypertension, hypercholesterolemia, diabetes mellitus, cardiovascular disease, cancer, deep venous thrombosis, asthma/COPD, rheumatoid arthritis, renal and/or liver disease.

Discussion

This large population-based study, comprising randomly selected adults of all age groups, provided the opportunity to investigate the influence of age on the relationship between thyroid function and mortality in a population in which TSH decreases and FT_4 increases with age.[30]

Regarding the relationship between thyroid function in euthyroid subjects and mortality, the association between higher FT_4 levels within the normal range and mortality was only present in the oldest participants. These findings are similar to the results of the Leiden 85+ study, the studies of Waring et al. and Van der Beld et al.[24, 27, 29] The reason why higher FT_4 but not lower TSH levels within the normal range predict mortality in this oldest group is not clear. As previously hypothesized, these results might suggest that there is a change of pituitary TSH set point in elderly, e.g. an altered pituitary sensitivity for thyroid hormones, and higher FT_4 levels do not cause the same TSH suppression as in younger individuals. Another explanation could be that higher FT_4 levels reflect a decreased 5'-deiodination due to non-thyroidal illness and are associated with lower T_3 levels. However, as shown by Waring et al. and in the Leiden 85+ Study, the relationship between FT_4 and mortality seems to be independent of the T_3 level.[24, 29] In our study, all the euthyroid subjects >80 years old with a FT_4 level of 18.5-22.0 pmol/L had a TSH level < 2.0 mIU/L. These subjects comprised only a small subset of the subjects with a TSH level < 2.0 mE/L. The majority of the subjects in this oldest group with a low-normal TSH had a low- or middle-normal FT_4 (8-18.5 pmol/L) and those subjects did not have a higher mortality. Therefore, FT_4 seems to be a better marker to predict mortality in elderly than TSH. On the other hand, a high-normal TSH but not a low-normal FT_4 level was also associated with mortality in elderly. Our study was a cross sectional survey and no causal relationships can be shown. Prospective intervention trials are needed to assess the targets for treatment in this subgroup.

Our study provides new insights for the ongoing debate about the upper limit of the reference range of TSH, especially in elderly. It has been suggested to increase the upper limit in elderly.[37, 38] Arguments for this recommendation are the increase of TSH with age in several (iodine sufficient) populations and the high amount of older, TPOAbs negative subjects with TSH above the upper limit of the currently used reference range.[29, 38, 39]. However, in our population TSH decreases and FT_4 increases with age probably due to mild iodine insufficiency in the past, leading to autonomous function of the thyroid (despite the adequate iodine status at present, achieved after increasing the amount of iodized salt in bakeries in 1982).[30, 40] Moreover, in our population, a high-normal TSH level was associated with mortality in elderly. So, the suggestion of increasing the upper limit of the reference range of TSH in elderly seems not appropriate

for our population. Reference ranges might be different not only for different races or ages, as previously suggested, but also for different populations. On the other hand, despite the fact that an increase of FT_4 with age appears to be 'normal' in our population (when using the population distribution to assess what is normal), we found that in elderly a higher FT_4 within the normal range was associated with mortality. This implicates that one should not only use the population distribution but also the clinical consequences of thyroid hormones levels to determine the reference ranges.

We found inconclusive and conflicting results regarding the relationship between TSH within the normal range and mortality in subjects aged 65-80 years old. These results do not seem to make sense from a biological point of view and it is possible that some of the significant results might be due to chance due to multiple testing. These findings reflect the discrepant results of previous studies investigating the relationship between thyroid function and mortality and endorse the current controversy regarding this topic.

As inevitable in a population based study, the number of subjects with subclinical and overt thyroid dysfunction was limited. Despite the small number of participants with subclinical thyrotoxicosis (n=193), we observed an association between subclinical thyrotoxicosis and mortality in younger subjects only, not in participants ≥ 65 years old. An explanation for this finding could be the fact that in older subjects there is a larger contribution of other cardiovascular risk factors, like age, and that there is more competing mortality (independent of thyroid function).

Similar to these results, meta analyses by Ochs et al. and Ravzi et al. reported no association between mortality and subclinical hypothyroidism in the total population, but they did find an association between subclinical hypothyroidism and mortality in younger participants (age <65 years).[17, 18] We could not detect a significant relationship between subclinical hypothyroidism and mortality. We cannot exclude that we had too limited power for this subgroup to show a modest relationship with mortality. Recently 6 meta-analyses regarding the relationship between thyroid function class and mortality reported conflicting results. Völzke et al. concluded that the current available evidence for a causal relation of thyroid dysfunction and mortality is weak due to highly discrepant results of previous studies, probably due to confounding and selection bias.[21] Singh et al. and Rodondi et al. found an association between cardiovascular mortality and subclinical hypothyroidism,[19, 20] whereas Haentjes et al. only found an association between subclinical hyperthyroidism and all cause mortality.[16] Ochs et al. and Ravzi et al. found no association between mortality and subclinical hypothyroidism in the total population, but they did find an association between subclinical hypothyroidism and mortality in younger

participants (age <65 years).[17, 18] These discordant results might be caused by varying methods of adjustment for confounders and the clinical heterogeneity among the studies due to differences in populations. As we have shown in this study, the age of the study participants can be of major influence on the outcome of the study and therefore might be one factor explaining the discrepancies between the studies.

Our study has a few limitations. We did not measure T_3, so we might have missed some cases of overt thyrotoxicosis in subjects with normal FT_4 and elevated T_3 and misclassified those subjects as having a subclinical thyrotoxicosis. Second, analyses were based on one single measurement of TSH and FT_4. Due to the variation of TSH and FT_4, the relationship between thyroid function and mortality might be underestimated as a result of regression dilution bias.[41] In addition, transient thyroid abnormality such as thyroiditis may have been misclassified. Third, at baseline we excluded subjects with known thyroid disease and those who used thyromimetic and/or thyrostatic drugs. However, we did not assess whether treatment with thyrostatic or thyromimetic drugs was started during follow up. Finally, we had no data on the causes of death so we could not perform cause specific mortality analysis.

In conclusion, age influences the relationship between thyroid function and mortality. Subclinical thyrotoxicosis was associated with mortality in younger subjects only. In elderly, within the normal range of thyroid function, both high-normal FT_4 and high-normal TSH levels were associated with mortality. The fact that previous studies comprised specific and different age groups, can, at least in part, explain the discrepant findings and the remaining controversy concerning the relationship between thyroid function and mortality.

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CHAPTER 5

Underestimation of effect of thyroid function parameters on morbidity and mortality due to intraindividual variation.

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Abstract

Introduction

Thyroid dysfunction is associated with several diseases and mortality. Due to the within-individual variation of TSH and FT_4 levels, the association between thyroid function and disease or mortality rates might be underestimated in studies based on one single measurement of TSH and/or FT_4. This kind of bias is called regression dilution bias. The aim of this study was to examine the within-individual variability of TSH and FT_4 measurements several years apart in different study cohorts and to determine the magnitude of the underestimation of the association between thyroid function and disease rates in population based studies using only one baseline measurement.

Methods

We used a pair of measurements of serum TSH and FT_4 levels of subjects of the Nijmegen Biomedical Study (NBS) and the Rotterdam Study (RS) to calculate the regression dilution ratio (RDR) with a nonparametric method, the Macmahon's method. Risk estimates could be corrected for regression dilution bias by dividing them by the RDR.

Results

The RDRs of serum TSH in the NBS and RS were 0.74 and 0.78, respectively. The RDRs of serum TSH were similar for women and men, 0.75 and 0.74 in the NBS and 0.80 and 0.77 in the RS. The RDR of serum FT_4 in subjects in the NBS was 0.77.

Conclusion

The relationship between thyroid function and disease rates is underestimated by studies using only one measurement of TSH and FT_4. The true association will be about 33% (1/0.75) higher for studies with a follow-up time of 2-4 years.

Introduction

Thyroid dysfunction is associated with several diseases, such as atrial fibrillation and cardiovascular disease, and with mortality.[1, 2] Most of the reported associations between thyroid function and disease or mortality rates in population-based studies are based on one single measurement of TSH and/or FT_4. These associations might be underestimated due to the intraindividual variation of the TSH and FT_4 measurements, which will especially be the case in studies with a long follow up period. This kind of bias is called regression dilution bias.[3-6]

The overall variation of the thyroid function measurement consists of several components. The preanalytical variation comprises the circumstances of blood withdrawal, like the time of the day, the use of tourniquet, whether the subject is fasting or rested or not. The analytical variation is another cause of variation but is expected to be smaller in comparison to the biological variation. The biological variation is caused by circadian variation, seasonal variation and other factors like change of age, weight, diet, smoking, pregnancy, medication and co-morbidity.[7-10]

The aim of this study was to examine the within-individual variability of TSH and FT_4 measurements several years apart in different study cohorts and to determine the magnitude of the underestimation of the association between thyroid function and disease rates in population-based studies using only one baseline measurement. In addition, we will illustrate this using an example.

Subjects and laboratory results

Subjects included in this study were derived from 2 large population-based studies. Approval to conduct the studies was obtained from the Institutional Review Board. We excluded the subjects with previously known thyroid disease, thyroid surgery, iodine treatment and/or the use of thyroid hormones or thyrostatic drugs in order to assess the variability of the thyroid function itself instead of the influence of thyrostatic and/or thyromimetic drugs or the clinical course of thyroid disease.

Participants of the Nijmegen Biomedical Study (NBS), a population-based survey performed in the eastern part of the Netherlands, are randomly selected, for age and sex stratified adults. From September 2002 until November 2003 non-fasting blood samples of 6434 subjects were taken. More details about this study are described elsewhere.[11] In 2005, subjects in the age group 50 through 70 years (N=2253) were invited to participate in a study on atherosclerosis. Of this subset of participants, 1161 subjects of the subjects consented and blood samples for

second TSH and FT_4 measurements were taken between June 2006 and April 2008. A total of 371 subjects were excluded because of previously known thyroid disease. Serum TSH was measured by an immunoluminometric assay on a random-access analyzer (Architect; Abbott Diagnostics Division). The reference interval used in the laboratory is 0.4-4.0 mIU/L. Serum FT_4 was measured with a luminescence enzyme immunoassay on a random-access assay system (Vitros ECI; Ortho Clinical Diagnostics). The laboratory reference interval is 8.0-22.0 pmol/L.

The Rotterdam Study (RS) is a prospective population-based cohort study of determinants of chronic disabling diseases in 7983 Caucasian elderly men and women from the western part of the Netherlands.[12] In a random selection of 1855 participants TSH measurements were done at baseline (October 1989 - June 1992). At the first follow-up visit (October 1993 - December 1994), second TSH levels were measured in 1562 participants. TSH levels were measured with a commercial TSH assay (Lumitest; Henning, Berlin, Germany [currently Brahms]). The reference range of TSH was 0.4-4.3 mU/L. A total of 145 subjects were excluded because of previously known thyroid disease. More details about this study are described elsewhere.[12]

Statistics

We used the pair of measurements of TSH and FT_4 to calculate the regression dilution ratio (RDR) with a nonparametric method, the Macmahon's method.[3-5] We first stratified the subjects of each study into quintiles, according to the value of the baseline measurement of TSH or FT_4. The initial range (R_1) was defined as the difference between the means of the top and bottom quintiles of the TSH or FT_4 measurements. Secondly, still using the baseline quintile stratification, the mean values of TSH or FT_4 of the second measurement were calculated per quintile and the range of the second measurements (usual range or R_2) was calculated.

Because of variation, the first measurement of some subjects will result in a higher value than the true or usual value. These subjects are more likely to be assigned to the highest quintile. Therefore, the upper quintile includes a disproportionately high amount of subjects whose baseline measurement happens to be somewhat higher than their usual value. The second measurement will generally result in a lower value in these subjects. As a result, the mean of the second measurements of subjects within the highest quintile will be lower in comparison to the mean of the first measurements. The opposite effect is present at the bottom quintile, resulting in a higher mean of the second measurements of the bottom quintile in comparison to the mean of the first measurements. Therefore, the range of the second measurements will generally be smaller than the baseline range.

The ratio of the two ranges, R_2/R_1, provides an estimate of the regression dilution ratio (RDR), which can be used as a measure of the extent of the variability. A measurement with a high variation will result in a lower second range and therefore have a low RDR. A measurement with no variation at all will result in an equal second range and therefore will have a high RDR of 1. A change of the mean of serum TSH or FT_4 during time in the population will decrease or increase all second quintiles, but will not have an effect on the range of the second quintiles and will not affect the RDR.

The RDR can be used to adjust the association between the baseline measurements and the disease rate. For example, one finds a relative risk (RR) of 2.0 for myocardial infarction for the upper versus lower quintile of the baseline cholesterol measurements. At baseline, the mean cholesterol levels of the upper and lower quintiles are 6 and 2 mmol/l respectively (range 4 mmol/L). Suppose at second survey, the mean cholesterol levels of the upper and lower quintiles are 5.5 and 2.5 mmol/L respectively (range 3 mmol/L), than the RDR =3/4. The true estimate is a relative risk of 2.0 for a 3 mmol/L difference instead of 2.0 for a 4 mmol/L difference. To express the relative risk for a true 4 mmol/L difference in cholesterol level one could multiply the regression coefficient (ln RR) by 4/3 (i.e. 1/RDR): the relative risk for a 4 mmol/L difference in cholesterol is 2.5 (exp(ln(2.0)/0.75), (the adjustment by using the RDR should be made on the natural logarithm of the relative risk instead of on the relative risk itself).

Results

Table 1 and figure 1 show the mean values of the log-transformed TSH and FT_4 values of each of the at baseline-defined quintiles by cohort. The RDRs of serum TSH in the NBS and RS were 0.74 and 0.78, respectively. The RDR of serum FT_4 in the NBS was 0.77. This means that the point estimate of an association in studies using one single measurement is underestimated by approximately 33% (1/0.75).

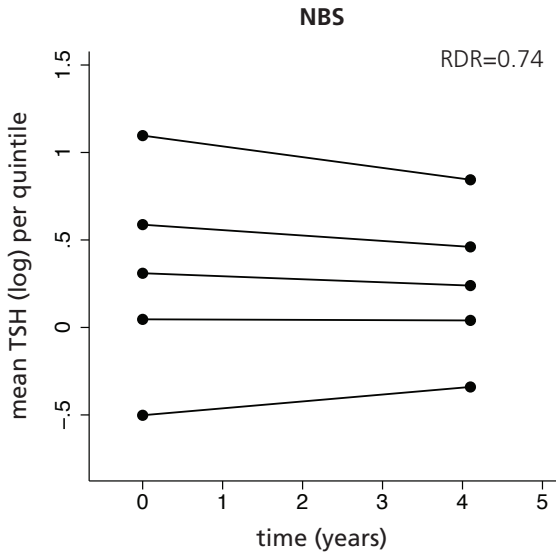
The RDRs of serum TSH were similar for women and men, 0.75 and 0.74 respectively in the NBS and 0.80 and 0.77 in the RS. The RDRs of serum TSH in participants, excluded because of known thyroid disease were 0.42 in the NBS and 0.52 in the RS.

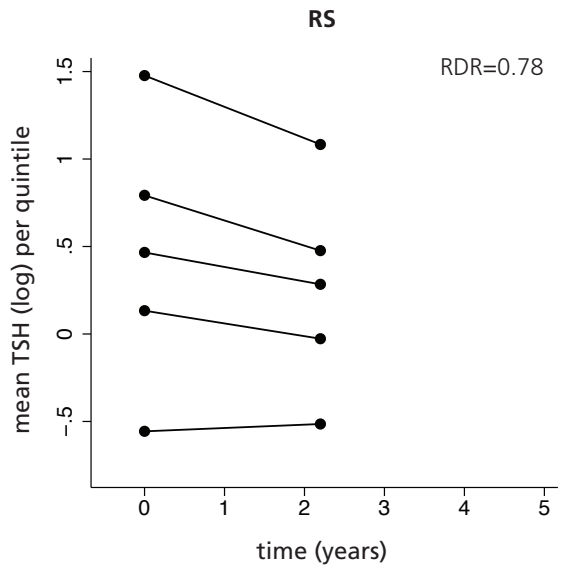
Table 1.
Mean TSH (log) and FT_4 per quintile and RDR

	NBS		**RS**	
	Baseline survey	**Second survey**	**Baseline survey**	**Second survey**
TSH quintile 1	-0.50	-0.34	-0.56	-0.51
TSH quintile 2	0.05	0.04	0.13	-0.03
TSH quintile 3	0.31 **1.59**	0.24 **1.18**	0.47 **2.04**	0.28 **1.59**
TSH quintile 4	0.59	0.46	0.79	0.48
TSH quintile 5	1.09	0.84	1.48	1.08
RDR	1.18/1.59**=0.74**		1.59/2.04**=0.78**	
FT_4 quintile 1	10.4	11.4	-	-
FT_4 quintile 2	12.0	12.6	-	-
FT_4 quintile 3	12.9 **5.7**	13.5 **4.4**	-	-
FT_4 quintile 4	13.9	14.3	-	-
FT_4 quintile 5	16.1	15.8	-	-
RDR	4.4/5.7**=0.77**			

RDR: regression dilution ratio (range second survey divided by range baseline survey).
NBS: Nijmegen Biomedical Study. RS: Rotterdam Study.

Figure 1.
Mean values of TSH (log-transformed) per quintile (based on the first measurements) at baseline and at second survey. RDR is range second survey divided by range baseline survey.





Discussion

Due to regression dilution bias, the association between thyroid function and disease rates or mortality rates in studies, based on one single measurement, is underestimated.[3-6] Due to an increase of variation over time, a longer period between baseline measurements and outcome assessment results in a higher degree of underestimation. The difference between short-term and long-term variation may be due to changes beyond chance variation, like change of age, weight, diet, smoking, medication and co-morbidity. In several long-term studies regarding the relationship between cardiovascular risk factors and cardiovascular disease, the underestimation is corrected for this bias using the RDR.[3, 13, 14] For example, the RDR of homocysteine was estimated 0.83 after 2 years and 0.71 after 6 years. The RDR after 10 years for cholesterol and blood pressure were estimated 0.65 and 0.52, respectively. (4;14) For the relationship between thyroid function and disease rates, most studies are based on a single measurement and do not address the point of underreporting the true risk due to intraindividual variability.

We estimated the degree of this underestimation by assessing the RDR of TSH and FT_4 in 2 large population-based studies in the Netherlands. There are several methods to estimate the RDR. We used the Macmahon's method. We choose for this method because of the simplicity and because of the relative short follow-up time.

We found that the RDRs for TSH were 0.74 and 0.78 in the NBS and the RS, respectively. This means that the estimated association in studies using one single measurement, is underestimated by approximately 33% (1/0.75). We can directly apply this approach on data of the RS concerning the relationship between TSH levels and atrial fibrillation. Heeringa et al. found an association between TSH level within the normal range and the risk of atrial fibrillation in the RS.[15] The published hazard ratio (HR) was 1.94, lowest versus highest quartile, based on one single measurement of TSH. The corrected HR for the baseline TSH levels of the lowest versus highest quartiles is actually 2.33 (this HR of 2.33 could be calculated as: exp(ln(1.94)/0.78)). One should be aware that this RS example is an simplified conceptual example, for an adjustment of estimates of multivariate models and for the calculation of confidence intervals we refer to the statistical literature. (16) In the NBS and the RS, the interval between the measurements was relatively short. The underestimation of the point estimates in studies using one single measurement is even more distinct in studies with a longer follow-up time.[3]

There are some limitations of the RDR corrections. The assumption is made that disease risk depends on a single underlying long-term exposure, instead of a time-dependent true underlying exposure. In studies with a follow-up of more than a few years, a 'life-course' approach is more appropriate. If the risk of disease is also dependent on past levels of TSH/FT_4, a 'non-life-course method' can lead to overcorrection of association.[16, 17] Another pitfall of correction with the RDR is the fact, that the observed TSH-disease association may reflect unmeasured confounding rather than a causal association. RDR corrections amplify such an association.

In conclusion, we found that associations between TSH/FT_4 and disease rates, estimated in studies using one single measurement, are underestimated by approximately one-third due to regression dilution bias. Clinicians and researchers should be aware that studies based on a single measurement of TSH or FT_4 do underestimate the true risk.

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CHAPTER 6

Longitudinal trends in thyroid function in relation to iodine intake: ongoing changes of thyroid function despite adequate current iodine status.

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Abstract

Objective

Several cross-sectional studies in populations with iodine deficiency showed that TSH-levels are negatively associated with age, while in populations with high iodine intake TSH is positively associated with age. The question is whether such an age-thyroid function relation is an ongoing process apparent also in longitudinal studies and whether it reflects an actual iodine deficiency or an iodine insufficiency in the past.

Methods

In an area with a borderline iodine status in the past we studied 980 participants of the Nijmegen Biomedical Study. We measured serum TSH, FT_4, total T_3, peroxidase antibodies and the urine iodine and creatinine concentration 4 years after our initial survey of thyroid function in which we reported a negative association between TSH and age.

Results

Within 4 years TSH decreased with 5.4% (95% CI 2,5%-8,3%) and FT_4 increased with 3.7% (95% CI 2.9%-4.6%). Median urinary iodine concentration was 130 µg/L. Estimated 24 hours iodine excretion was not associated with TSH, T_3, change of TSH or FT_4 over time or with the presence of TPOAb. Only FT_4 appeared to be somewhat higher at lower urine iodine levels: a 1.01% (95% CI 0.17-1.84) higher FT_4 for each lower iodine quintile.

Conclusions

In this longitudinal study, we found an ongoing decrease of TSH and increase of FT_4 in a previously iodine insufficient population, despite the adequate iodine status at present. This suggests that low iodine intake at young age leads to thyroid autonomy (i.e. a tendency to hyperthyroidism) that persists despite normal iodine intake later in life.

Introduction

Iodine is an essential micronutrient and an important component of thyroid hormones. Iodine deficiency can cause thyroid dysfunction, goiter and cretinism.[1] Monitoring the iodine status and maintaining an optimal iodine intake is very important to prevent brain damage in newborns and thyroid function disorders at all ages. Iodine deficiency remains a global public health problem.[2] To assess the iodine status of a population, the median iodine concentration of series of single urine samples is the most widely used measurement.[1, 3] In large population studies, there is a leveling out of the day-to-day variation and the median value of the urinary iodine concentration in samples can be used to assess whether a population is iodine sufficient.[4] According to the World Health Organization (WHO) criteria, a population has an optimal iodine intake if its median urine iodine concentration is between 100 and 199 µg/L and no more than 20% of the population has an urinary iodine concentration of 50 µg/L or less.[1, 3]

In the past, mild iodine deficiency was present in the eastern and southern part of the Netherlands.[5-7] Iodine supplements were instituted as of 1935. Since then, several additional measures, like the compulsory use of iodized salt in bakeries, instituted in 1963, were taken to achieve a daily intake of iodine within the optimal range as recommended by the WHO.[3] Currently, the iodine status of the Netherlands is considered to be adequate, based on studies regarding the iodine intake and urinary excretion in several regions in the Netherlands.[2, 8-12] Due to decrease of salt consumption and reduced bread consumption, regular monitoring of the iodine status in the population is necessary to verify the maintenance of the adequate iodine status.[13]

Previous cross sectional population studies have shown that in populations with a history of mild or moderate iodine deficiency, the average serum level of thyroid stimulating hormone (TSH) is negatively associated with age and free T_4 (FT_4) is positively associated with age, which is probably due to the gradual development of autonomous function of the thyroid gland.[14-16] By contrast, in populations with high iodine intake, TSH is positively associated with age.[17] In subjects of the Nijmegen Biomedical Study (NBS), a large population-based survey performed in Nijmegen, a municipality in the eastern part of the Netherlands, TSH is negatively associated with age and FT_4 is positively associated with age.[14] These associations were found in cross-sectional surveys. The question is whether such an age-thyroid function relation is apparent also in longitudinal analyses and, if so, whether it reflects an actual iodine deficiency.

Materials and Methods

Subjects

The subjects of this study are a subset of the participants of the Nijmegen Biomedical Study (NBS), a large population-based survey performed in Nijmegen, a town in the eastern part of the Netherlands. Details of this study have been described previously.[14] Approval to conduct the study was obtained from the Institutional Review Board of the Radboud University Nijmegen Medical Centre (RUNMC). A total of 2253 respondents, aged 50 to 72 years, were invited to participate in a study of non-invasive measurements of atherosclerosis (NBS-NIMA) of whom 1517 gave their informed consent. In 1052 participants of this subgroup, the iodine concentration was measured in fasting morning urine samples in 2006. Serum TSH and FT_4 were measured twice with an interval of 4 years (2002 and 2006). The mean interval between the measurements was 4.1 years (range 2.8-5.4 years). Triiodothyronine (T_3) and antibodies against thyroid peroxidase (TPOAb) were measured in 2006. We excluded subjects with previously known thyroid disease, a medical history of thyroid surgery or iodine treatment, the use of thyromimetic and/or thyrostatic drugs and the use of medication interfering with thyroid function or iodine status such as amiodarone, kelp, oral corticosteroids, dopamine agonists and lithium. A total of 72 participants were excluded, so in total 980 participants were included in the current analysis.

Laboratory methods

Serum TSH and T_3 were measured by an immunoluminometric assay on a random-access analyzer (Architect; Abbott Laboratories, Abbott Park IL 60064, USA). The reference interval for serum TSH used in our laboratory is 0.4-4.0 mIU/L. Serum FT_4 was measured with a luminescence enzyme immunoassay on a random-access assay system (Vitros ECI; Ortho Clinical Diagnostics, Rochester, NY, USA). Our laboratory reference interval is 8.0-22.0 pmol/L. TPOAbs were measured with a fluorescence immunoenzymometric assay for the quantitative measurement of the IgG class of anti-thyroperoxidase antibodies (AxSYM, Abbott Laboratories, Abbott Park IL 60064, USA). The reference interval was defined as <12 kIU/L (data provided by manufacturer). More details about these measurements are described elsewhere.[14] In order to control a possible drift in assays between the 2 study periods, we repeated TSH and FT_4 measurements of pooled plasma with TSH and FT_4 in the lower, middle and higher range. For TSH, the control samples showed a drift of -2.2, -6.0, and -9.6% for the lower, middle and higher range, respectively. For FT_4, the control samples showed a drift of -9.7, -5.7 and -1.8% for the lower, middle and higher range, respectively. We suspected that a bias was introduced during analysis of the control samples, perhaps by sample instability in the freezer or some other unknown factor. Therefore, we choose to use the original results of the assays.

To assess the iodine status of our population, we calculated the median urinary iodine concentration, obtained from single urinary samples, as recommended by the WHO.[1, 3] For the individual subjects, we used the estimated 24-hour iodine excretion, adjusted for age and gender, as an indicator for the iodine status. The estimated 24-hour urinary iodine excretion was calculated as follows: iodine (µg/L)/creatinine (g/L) x expected 24-hour creatinine (g/day).[18-20] A large Belgian population study provides data on the expected creatinine excretion per individual, taking age and sex into account.[21] The urine iodine concentration was measured with the Ammonium Persulfate Destruction Microplate (APDM) method, using a Peltier Thermal Cycler (PTC-200) for the heating and cooling process.[22] The urinary creatinine concentration was measured with an enzymatic assay (Roche, Basel, Switzerland) on the Aeroset chemistry analyser (Abbott Laboratories, Illinois, USA).

Statistical analysis

Because of a skewed distribution of the urinary iodine concentration, we used quintiles of the estimated 24-hour urinary iodine excretion for stratification. We displayed geometric means of FT_4 and TSH with its 95% confidence intervals. We expressed change over time of TSH and FT_4 as a percentage of change. We performed logarithmic transformation of and TSH for regression analysis. Linear regression analysis was performed in order to investigate the relationship between thyroid function and iodine excretion. Each model included the estimated 24-hour urine iodine excretion as the independent variable and a thyroid function parameter (either FT_4, T_3, TSH or change of TSH or FT_4 over time) as the dependent variable. To control for possible confounding we added age, gender, BMI and current smoking status to the models. We analysed the data with STATA version 11.0 (StataCorp, Texas).

Results

The characteristics of the population are shown in table 1. The median urinary iodine concentration was 130 µg/L. The median urinary iodine concentration was higher in men in comparison to women, 156 µg/L versus 104 µg/L respectively. The urinary iodine concentration was less than 50 µg/L in 15% of the participants. The urinary iodine concentration was less than 50 µg/L in 9% of the men and in 20% of the women. Due to a higher urinary creatinine concentration, men had a lower urinary iodine/creatinine ratio in comparison to women (135 µg/g versus 171 µg/g, respectively). However, the median estimated 24-hour urinary iodine excretion, taking age and gender into account, was higher in men in comparison to women (208 µg/day versus 186 µg/day, respectively).

Table 1.
Population characteristics.

	Total		**Men**		**Women**	
Number	980		492		488	
Mean age (range), years	61	(50 to 72)	62	(50 to 72)	61	(50 to 71)
Serum TSH in 2002 (mIU/L)[a]	1.35	(1.30 to 1.41)	1.35	(1.28 to 1.42)	1.35	(1.27 to 1.43)
Serum TSH in 2006 (mIU/L)[a]	1.29	(1.24 to 1.34)	1.30	(1.24 to 1.36)	1.28	(1.20 to 1.36)
Change of TSH over time[b]	-5.4%	(-8.3 to -2.5%)	-5.5%	(-8.6 to -2.4%)	-5.3%	(-10.0 to 0.3%)
Serum FT_4 in 2002 (pmol/L)[a]	13.0	(12.9 to 13.1)	13.0	(12.8 to 13.2)	12.9	(12.8 to 13.1)
Serum FT_4 in 2006 (pmol/L)[a]	13.5	(13.3 to 13.6)	13.4	(13.2 to 13.6)	13.5	(13.3 to 13.8)
Change of FT_4 over time[b]	3.7%	(2.9 to 4.6%)	2.9%	(1.7 to 4.2%)	4.5%	(3.3 to 5.8%)
Serum T_3 (nmol/L)[a]	1.61	(1.59 to 1.62)	1.61	(1.59 to 1.63)	1.60	(1.58 to 1.62)
Presence of TPOAb (%)[c]	115	(11.9%)	27	(5.6%)	88	(18.2%)
Urinary Iodine conc (µg/L)[d]	130	(70 to 211)	156	(91 to 238)	104	(55 to 176)
Urinary Creatinine conc (g/L)[d]	0.89	(0.4 to 1.5)	1.2	(0.7 to 1.8)	0.6	(0.3 to 1.0)
Iodine/Creatinine ratio (µg/g)[d]	154	(109 to 212)	135	(101 to 186)	171	(120 to 241)
Estimated 24-hour urinary iodine excretion (µg/day)[d]	199	(145 to 274)	208	(151 to 287)	186	(134 to 262)

TSH, thyroid stimulating hormone; FT_4, free T_4; TPOAb, antibodies against thyroid peroxidise; Conc, concentration.
[a] Geometric means (95% CI)
[b] Percentage change of TSH and FT_4 from 2002 until 2006, geometric means (95% CI)
[c] TPOAb missing in 14 subjects
[d] Median (interquartile range)

During the follow up period of 4 years, the average TSH decreased with 5.4% (95% CI 2.5% to 8.3%, p-value <0.001) and FT_4 increased with 3.7% (95% CI 2.9% to 4.6%, p value <0.001) (Table 1).

A lower quintile of estimated 24-hour iodine excretion was associated with a 1.01% higher FT_4 (95% CI 0.17 to 1.84, p for trend along quintiles 0.02, adjusted for age, gender, BMI and current smoking status). There was no significant association between the TSH level and the estimated 24-hour iodine excretion after adjustment for age, gender, BMI and current smoking status: a lower quintile of urinary iodine excretion resulted in a 0.17% higher serum TSH (95% CI -3.0 to 3.2, p for trend along quintiles 0.92). There was no association between T_3 and the estimated 24-hour iodine excretion.

There was no association between iodine excretion and the percentage of decrease of TSH or increase of FT_4: a higher quintile of the estimated 24-hour iodine excretion resulted in a 1.4% stronger decrease of TSH over time (95% CI -1.0 to 3.6, p for trend along quintiles 0.25 after adjustment for age, gender, BMI and current smoking status) and a lower quintile of the estimated 24-hour iodine excretion resulted in a 0.4% stronger increase of FT_4 over time (95% CI -0.3 to 1.0, p for trend along quintiles 0.26 after adjustment for age, gender, BMI and current smoking status).

There was no relationship between the presence of TPOAb and estimated 24-hour urinary iodine excretion (data not shown). After exclusion of subjects with TPOAb, the analyses of TSH, FT_4 and T_3 gave similar results (data not shown). Analyses with TSH or FT_4 and iodine as (logtransformed) continuous variables gave similar results (data not shown).

Discussion

Despite the fact that our population has an optimal iodine intake at this moment, we found an ongoing decrease of TSH and increase of FT_4 over time. The decrease of TSH and the increase of FT_4 in the 4 years follow up period was even larger than we expected, based on the cross-sectional data in 2002. Iodine excretion was in the recommended range and was not associated with a change in TSH or FT_4. One might speculate that the decrease of TSH and increase of FT_4 with age in our population is caused by mild iodine deficiency in the past and not by iodine deficiency in the present. Iodine deficiency in the past may have led to autonomous thyroid function, which at present causes an ongoing increase of FT_4 and decrease of TSH over time.

The mechanism by which mild iodine insufficiency leads to thyroid autonomy is only partially understood. In case of mild iodine deficiency, low iodine intake might lead to a reduced T_4 and T_3 production. In order to prevent this, several TSH-independent autoregulatory mechanisms are triggered, such as an increase in vascularity, an increase in iodine uptake and increased T_3 production and secretion, at the expense of T_4.[23] If these mechanisms fail, TSH levels will rise as a response to a lower thyroid hormone production. TSH stimulates follicular cell replication and due to the higher replication rate, the chance of activating mutations in the TSH-receptor gene leading to TSH-independent growth and function, is increased.[24] This and other mechanisms lead to autonomous function of the thyroid and, especially when iodine intake is supplemented, to hyperthyroidism.

We found that a lower estimated 24-hour iodine excretion was associated with a somewhat higher serum FT_4. However, we found no relationship between TSH and the estimated 24-hour urinary iodine excretion. Previous studies reported conflicting results on the relationship between urinary iodine excretion and thyroid function. Haddow et al. found no relationship between urine iodine concentration and thyroid function and only a weak positive association between the urinary iodine/creatinine ratio and TSH.[25] Their study was performed in an iodine sufficient population, where TSH increases with age.[17] Hwang et al. found that the urinary iodine/creatinine ratio had a negative correlation with FT_4 and showed a positive trend with TSH in a Korean population, with a high iodine intake.[26] In this Korean study, creatinine adjustment of urine iodine measurements was done without taking gender and age into account.

We used the estimated 24-hour iodine excretion, adjusted for age and gender, as an indicator for the iodine status of individuals. Assessing the iodine status of individuals is difficult. The within-day and day-to-day variation in urinary iodine excretion is large.[27] Therefore, repeated 24-hour urine samples are considered to be the best measure.[28] However, 24-hour collections

are not very practical to perform when large numbers of persons have to be investigated. To minimize the variation in urinary iodine concentration caused by a variable urinary volume, the iodine / creatinine ratio (I/C ratio) has also been used to assess the iodine status. However, creatinine excretion varies with sex, age, cultural and genetic background. That is the reason why adjustment for age and sex is recommended.[18, 19, 27, 28] This can be done by estimating the 24-hour iodine excretion, using the expected 24-hour creatinine. A large Belgian population study provides data on the expected creatinine excretion per individual, taking age and sex into account.[21]

Our study has a few limitations. Our study is a subset of a large epidemiological study, the NBS-NIMA study. Because of the nature of the NBS-NIMA survey, urinary iodine measurements were only performed in subjects aged between 50 and 72 years old. Second, as already mentioned, assessing the iodine status of an individual is difficult due to variation of iodine intake and excretion. Due to the variation in iodine excretion, the relationship between iodine excretion and thyroid function parameters might be underestimated as a result of regression dilution bias.[29-31] Third, the fact that sera from 2002 and 2006 were assayed several years apart introduced a potential bias. Although this potential bias by variation in assay is inescapable in every large population study using biochemical analyses at different moments, this problem is not always recognized and addressed. Finally, although previous population studies showed a iodine insufficiency in the past in this part of the Netherlands, we have no historical data on iodine excretion of this cohort to support our hypothesis that the current changes in thyroid function are due to iodine insufficiency in the past.[5-7]

In conclusion, in this longitudinal study, we found an ongoing decrease of TSH and increase of FT_4 in a previously iodine insufficient population, despite the adequate iodine status at present. This suggests that low iodine intake at young age leads to thyroid autonomy (and a tendency to hyperthyroidism) that persists despite normal iodine intake later in life.

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CHAPTER 7

Thyrotropin versus age relation as an indicator of historical iodine intake.

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Abstract

Background

In populations with mild iodine deficiency, serum level of thyrotropin (TSH) is negatively and serum free T_4 (FT_4) is positively associated with age. An ongoing decrease of TSH and increase of FT_4 can be found after supplementation of iodine. The aim of this study was to investigate whether there are current differences in the relation between thyroid function and age in relation to differences in iodine intake in the past.

Methods

Eight medical laboratories in several regions of the Netherlands, which are all iodine sufficient at present, but with a difference in iodine status in the past, provided the results of all TSH and FT_4 measurements performed from 2006 until 2011, resulting in 330,802 TSH and 103,940 FT_4 measurements.

Results

The negative association between TSH and age in elderly is only present in areas with a historically iodine deficiency (RC -0.008, 95% CI -0.009;-0.007). In the historical iodine sufficient population, TSH shows no obvious increase or decrease with age. In both the historically iodine sufficient and deficient populations, FT_4 levels were positively associated with age in elderly (RC 0.009, 95% CI 0.008;0.010 and RC 0.008, 95% CI 0.007;0.010 respectively).

Conclusions

There are differences in relation between thyroid function and age between populations with differences in iodine intake in the past, despite an adequate iodine status at present. This raises the question whether the present but also historical iodine status of a population should be taken into account when establishing the reference limits of TSH and FT_4.

Introduction

Iodine is an important component of thyroid hormones. Severe iodine deficiency can cause primary hypothyroidism and cretinism. In case of mild iodine deficiency, several autoregulatory mechanisms within the thyroid are triggered to prevent hypothyroidism.[1] When these mechanisms fail, there is an increase of thyrotropin (TSH) secretion through hypothalamic/pituitary feedback in order to maintain euthyroidism. The compensatory mechanisms within the thyroid and the continuous TSH stimulation eventually may lead to growth and autonomous function of the thyroid gland and, especially when iodine is supplemented later, to hyperthyroidism.

Some large population based studies, such as the NHANES III study, reported that serum TSH level is positively associated with age in populations with adequate or high iodine intake.[2-6] The fact that elderly have on average higher TSH values in these iodine sufficient populations is one of the reasons why it has been suggested to increase the upper reference limit of normal serum TSH in elderly.[7, 8] However, other cross-sectional population studies have shown that in populations with mild or moderate iodine deficiency, the average serum level of TSH is negatively and that of free T_4 (FT_4) positively associated with age, probably due to autonomous function of the thyroid gland.[9-12] The suggestion of increasing the upper limit of the reference range of serum TSH in elderly seems therefore not appropriate for these populations.

Knudsen et al. showed a different association between serum TSH level and age in two areas in Denmark with slightly different iodine status.[10] In Aalborg, an area with moderate iodine insufficiency, there was a decline in serum TSH levels with age, whereas in Copenhagen, an area with only a very mild iodine insufficiency, this decline was not present. So, even within a relatively small geographic area, differences of iodine intake seem to influence the relationship between thyroid function and age.

In the past, mild iodine deficiency was present in the eastern and southern part of the Netherlands.[13-15] After the introduction of the water supply system (around 1900), the prevalence of goiter increased in the eastern and southern part of the country. The ground water that was used for the water supply system contained less iodine than the previously used more superficial ground water and contained less iodine in the eastern and southern part of the Netherlands than in other parts of the Netherlands.[15] Iodine supplementation was instituted as of 1935. Since then, several additional measures, like the compulsory use of iodized salt in bakeries, instituted in 1963, were taken to achieve a daily intake of iodine within the optimal

range as recommended by the World Health Organization (WHO).[16] Despite these efforts at increasing iodine intake, in 1981 the iodine status was still insufficient in the eastern part of the Netherlands and goiter was more prevalent in comparison to the western part of the Netherlands.[15, 17] Therefore, in 1982, the amount of iodized salt in bakeries was increased. Currently, the iodine status of the Netherlands is considered to be adequate, based on studies regarding iodine intake and urinary excretion in several regions in the Netherlands.[18-24]

In subjects of the Nijmegen Biomedical Study (NBS), a large population-based survey in the eastern part of the Netherlands, serum TSH level is negatively associated with age and FT_4 level is positively associated with age.[12] Despite the adequate iodine status of this population at this moment, a currently ongoing decrease of TSH levels and increase of FT_4 levels in subjects 50-72 years old was found in a longitudinal study.[25] The decrease of TSH and increase of FT_4 with age in this population is probably caused by the mild iodine deficiency in the past. Iodine deficiency in the past may have led to autonomous thyroid function, which at present causes an ongoing increase of FT_4 levels and decrease of TSH levels over time.

These studies suggest therefore that both the current and the past iodine intake can influence the relationship between thyroid function and age. Because iodine insufficiency in the past seems to cause an ongoing increase of FT_4 and decrease of TSH even after attaining an adequate iodine status, we hypothesized that there might also be differences in the relationship between thyroid function and age in populations that had a different iodine intake in the past. Therefore, the aim of this study was to investigate the relation between thyroid function and age in populations from several geographic regions in the Netherlands which are iodine sufficient at present but had a different iodine intake in the past.

Materials and Methods

To sample several regions with different iodine intake in the past we used routinely achieved TSH and FT_4 levels from laboratories that perform these measurements ordered by general practitioners for screening purposes. Because thyroid hormone assays are ordered with high frequency we hypothesized that the results are representative for the regional population. Eight large medical laboratories were invited to participate. The laboratories were chosen for their geographic localization in the Netherlands, namely either in the historically iodine deficient southern/eastern part of the Netherlands or in the historically iodine sufficient western part of the Netherlands.[15] The participating laboratories were situated in the cities

Nijmegen, 's-Hertogenbosch, Doetinchem, Helmond, Breda, Haarlem, Amsterdam and Leiden. They each provided the results of all TSH and FT_4 measurements performed at the respective laboratories from 2006 until 2011 (the measurements from Leiden were performed in 2013 only, the measurements from Amsterdam were performed from 2006 until 2013). For each person in whom TSH or FT_4 level was measured, data on age and gender were provided. All measurements of TSH and FT_4 were performed on request of a general practitioner in outpatients. Serum TSH (3rd generation assay) and FT_4 levels were measured by random access analyzers (ECLIA Modular E170, Roche; LEIMA Cobas 6000, Roche; ECLIA Dimension Vista, Siemens; LEIMA Immulite 2000, Siemens; CLIA DxI800, Beckman Coulter). Details of the participating laboratories and the laboratory methods are shown in Supplementary Table 1.

To ensure that we included only the treatment naïve patients we applied the following strategy. We assumed that when thyroid hormone levels of a patient without previously known thyroid disease are measured, this is probably because of symptoms like fatigue or weight gain etc. Most of the time, the general practitioner will also order some other blood analyses in order to screen for common diseases in such symptomatic patients. Some of the participating laboratories offer a package of several blood analyses to general practitioners to screen for some common causes of fatigue in patients. These packages for example include glucose level, erythrocyte sedimentation rate, hemoglobin level and TSH level. When such package was available at a laboratory, only these TSH measurements were included. If such package was not available, only TSH measurements were included if glucose and hemoglobin level were measured at the same time. In one laboratory (Helmond), glucose levels were not available, but hemoglobin levels were required for inclusion. We assumed that most patients using thyroid medication get their thyroid hormones level monitored periodically by measuring TSH and/or FT_4 levels only. Therefore, we excluded all subjects in whom only a serum TSH and/or a FT_4 level was measured.

Historical data of iodine status in the regions of the participating laboratories are shown in Table 1. In Haarlem, Leiden and Amsterdam, three cities situated in the western part of the Netherlands, low goiter prevalence has been reported in the past (1951) whereas a high goiter prevalence has been reported in the cities in the eastern/southern part of the Netherlands.[26] Iodine intake was higher in Leiden in the late seventies in comparison to some cities (including Helmond and Nijmegen) in the eastern/southern part of the Netherlands.[15] In the early eighties, iodine intake was still insufficient in women (in Doetinchem, Helmond but also in Leiden).[13] For the past 20 years, a sufficient iodine intake has been reported in all regions of the Netherlands.[18-24]

Table 1.
Overview of historical data on iodine status of participating regions.

	Geographic region in The Netherlands	Historical data on iodine status
Nijmegen	Eastern part	1951 goiter prevalence 40-60%[26]
		1977 urinary iodine excretion 80 µg/day, goiter prevalence 47%[15]
		2006 median urinary iodine concentration 130 µg/l[25]
's-Hertogenbosch	Southern part	1951 goiter prevalence 20-30%[26]
Helmond	Eastern-southern part	1951 goiter prevalence 40-60%[26]
		1987 urinary iodine excretion ♀111-♂135 µg/day, goiter prevalence 31-39%[13]
Doetinchem	Eastern part	1951 goiter prevalence >60%[26]
		1977 urinary iodine excretion ♀69-♂118 µg/day, goiter prevalence 20-50%[15]
		1987 urinary iodine excretion ♀122-♂157 µg/day, goiter prevalence 20-35%[13]
		1995 median urinary iodine concentration 151-166 µg/l, goiter prevalence 0.8%[20]
		2006 urinary iodine excretion 236 µg/day[24]
		2010 urinary iodine excretion 165 µg/day[24]
Breda	Southern part	1951 goiter prevalence 40-60%[26]
Haarlem	Western part	1951 goiter prevalence 0-20%[26]
		1995 median urinary iodine concentration 142-168 µg/l, goiter prevalence 2.6%[20]
Amsterdam	Western part	1951 goiter prevalence 20-30%[26]
		1995 median urinary iodine concentration 142-168 µg/l, goiter prevalence 2.6%[20]
Leiden	Western part	1951 goiter prevalence 20-30%[26]
		1977 urinary iodine excretion ♀123-♂126 µg/day, goiter prevalence 8-10%[15]
		1987 urinary iodine excretion ♀114-♂153 µg/day, goiter prevalence 7-32%[13]

Statistical analysis

We standardized the TSH and FT_4 values in order to show the results of different laboratories combined, using z scores (the number of standard deviations that a value differs from the mean). Because of a skewed distribution of TSH, TSH measurements were (natural) log transformed and we displayed geometric means of TSH with its 95% confidence intervals when presenting the results per laboratory separately (supplementary data). Linear regression analysis was used in order to describe the association between thyroid hormones (z scores, TSH after logtransformation) and age. Because of a non-linear relationship between thyroid hormones and age, as shown by figure 1, the population was divided in different age categories (0-35; 35-60; 60 years or older) and linear regression analyses was performed per age category. In order to compare the regression coefficients (RC), we included an interaction term in the regression model.

Results

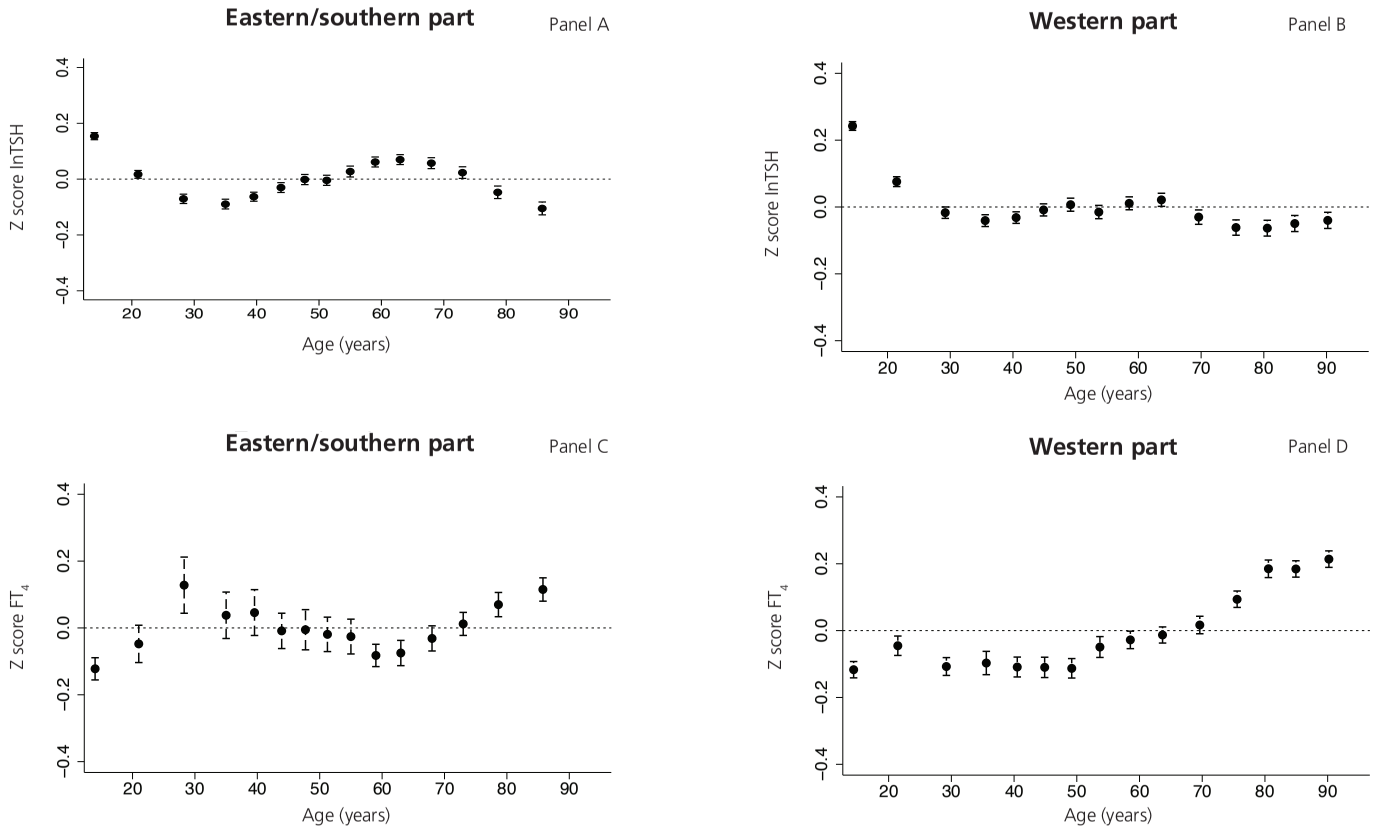
In total, 894,435 TSH measurements and 448,655 FT_4 measurements were obtained. A total of 330,802 TSH and 103,940 FT_4 measurements were performed in combination with (glucose and) hemoglobin measurements and these results were used for analyses. The numbers of included TSH and FT_4 measurements per laboratory are shown in Supplementary Table 1.

Figure 1 shows the standardized values of TSH and FT_4 for the eastern/southern part ('s-Hertogenbosch, Nijmegen, Doetinchem, Helmond and Breda) and the western part of the Netherlands (Amsterdam, Leiden and Haarlem). In both the eastern/southern part and the western part, average TSH levels are negatively associated with age during young adulthood. In the eastern/southern part, average TSH levels then increase gradually from approximately 35 years until the age of 60 years. In the elderly (60 years or older), average TSH levels decrease with age (RC -0.008, 95% CI -0.009;-0.007, this corresponds with a decrease of 0.7% of the average TSH level per year). In the western part, TSH shows no obvious increase or decrease with age in the population of approximately 35 years and older (age 60 years or older: RC -0.002, 95% CI -0.003;-0.001, this corresponds with a decrease of 0.1% of the average TSH level per year). The effect size is only very small and when considering figure 1, we conclude that there is no obvious trend of decrease or increase of TSH in elderly in the western part. The difference in RC between the eastern/southern part and western part in elderly is 0.006, 95% CI 0.004-0.007, $p<0.001$.

The average FT_4 levels in the eastern/southern part show the opposite pattern of the TSH levels in this area: the FT_4 levels are positively associated with age during young adulthood and then decrease until the age of 60 years. In subjects aged 60 years or older, FT_4 levels further increase with age (RC 0.008, 95% CI 0.007-0.010, this corresponds with an increase of 0.04 pmol/L of the average FT_4 level per year). In the western part, FT_4 levels show no obvious increase or decrease with age until the age of 60 years. In subjects, 60 years or older, FT_4 levels are positively associated with age (RC 0.009, 95% CI 0.008;0.010, this corresponds with an increase of 0.04 pmol/L of the average FT_4 level per year).

Supplementary Figure 1 shows the geometric means of TSH levels by age per laboratory. Supplementary Figure 2 shows the means of FT_4 levels by age per laboratory.

Figure 1.
Standardized values of serum TSH (panel a) and FT_4 (panel c) for the eastern/southern part (historical iodine deficient areas: 's-Hertogenbosch, Nijmegen, Doetinchem, Helmond and Breda) and the western part of the Netherlands (panel b+d) (historical iodine sufficient areas: Amsterdam, Leiden and Haarlem). Total number of measurements: serum TSH eastern/southern part 178,074; western part 152,728; serum FT_4 eastern/southern part 26,297; western part 77,643. TSH, thyrotropin; FT_4, free thyroxine.



Discussion

The aim of this study was to investigate whether there are differences in the relation between thyroid function and age between populations with differences in iodine intake in the past, despite an adequate iodine status at present, indicating that the TSH/FT_4 versus age relationship could be used as an indicator of historical iodine status. Our results show that the negative association between TSH and age in elderly (60 years or older) is only present in areas with a history of (mild) iodine deficiency. In the areas with a historical more adequate iodine status, TSH levels were neither positively nor negatively associated with age. An explanation for these findings could be the fact that due to mild iodine deficiency in the past, several compensatory mechanisms within the thyroid and continuous TSH stimulation have led to growth and autonomous function of the thyroid. When thereafter iodine intake is supplemented, a tendency to hyperthyroidism may develop. Another explanation could be that iodine deficiency has an epigenetic effect early in life, perhaps even prenatally, which is persistent despite of iodine repletion.

Our results agree with those of Knudsen et al, which showed a different association between TSH and age in two areas in Denmark with slightly different iodine status at present.[10] In our study, in the previously iodine deficient area, we could detect the negative association between TSH and age in elderly (60 years or older) only. By contrast, in younger subjects (aged 35-60 years old), TSH was positively associated with age, causing a remarkable curve in the TSH-age relationship figure. The reason for this might be the fact that the younger subjects are not exposed to iodine deficiency in the past, due to the mandatory use of iodized salt in bakeries in the Netherlands since 1963 and the successive efforts of the Dutch government to achieve an adequate iodine status since then. Therefore, the younger subjects are less likely to have developed growth and autonomous function of the thyroid. We hypothesize that if one would examine the relationship between TSH and age in several decades in this area, one might find a positive association between TSH and age in all age groups 35 years or older. Probably the results would then be more in line with the results of the NHANES III study, a large population study in an iodine sufficient area, which reported a positive association between TSH and age in all age groups.[2]

In the historical iodine deficient area, FT_4 levels are negatively associated with age in subjects aged 35-60 years and positively associated with age in the young subjects aged 35 years or younger and in the elderly, aged 60 years or older, i.e. the exact opposite of the associations between TSH levels and age in this area, as one would expect. However, despite the lack of a positive or negative association between TSH levels and age in the historical iodine sufficient

area, FT_4 levels were positively associated with age in elderly in this population. It has been previously hypothesized that there is a change of pituitary TSH set point in elderly and higher FT_4 levels do not cause the same TSH suppression as in younger individuals.[4, 5] Possible mechanisms for this are an altered pituitary sensitivity for thyroid hormones, a decrease in TSH bioactivity and/or a decreased responsiveness of the thyroid gland to TSH in elderly. The studies of Bremner et al. and Waring et al. showed in iodine sufficient populations an increase of TSH with age without decrease or even a small increase of FT_4 levels with age in elderly.[4, 5]

The fact that elderly have on average higher TSH values in these two study populations and in several other iodine sufficient populations is one of the reasons why it has been suggested to increase the upper reference limit of TSH level in elderly.[4, 5, 7, 8] However, the present study and other studies reporting a lower average TSH in elderly in current or historical iodine insufficient populations raise the question whether, besides age and race, the present and historical iodine status of a population should be taken into account when establishing the reference limits of TSH and FT_4.[9-12] Guan et al. compared three areas with different levels of iodine intake and showed that the average TSH level and the 2.5th and 97.5th percentiles were lowest in the iodine deficient area and highest in the population with iodine excess, even in a rigorously selected reference population after exclusion of subjects with thyroid disease, thyroid antibodies, thyroid nodules/goiter or a family history of thyroid disease.[27]

In our study there was a striking negative association between TSH and age and the concordant positive association between FT_4 and age in the young subjects (aged 35 years and younger). In particular the negative association between TSH and age was a consistent finding in all the laboratories. Most population-based studies include adults only, so little is known about the relationship between thyroid function and age in children and teenagers. The population study of Guan et al. shows similar results: the mean TSH level in the youngest age group (14–19 years old) was about 20%–30% higher than those in other age groups, even after exclusion of subjects with thyroid disease, thyroid antibodies, thyroid nodules/goiter or a family history of thyroid disease.[27] Further studies, preferably performed in an iodine sufficient population and after exclusion of subjects with thyroid disease and risk factors for thyroid disease, are needed to confirm these interesting findings.

A limitation of our study is the fact that we had no data on medical history of thyroid disease or thyroid autoimmunity of the participating subjects. In order to address this problem, we included only the subjects in whom a screening package of several laboratory measurements was performed and assumed that these subjects were less likely to have a known thyroid disease. Subjects on thyroid medication more likely would have their thyroid hormones level

monitored periodically by measuring TSH (and/or FT_4) levels only. The results of the laboratory of Nijmegen, that offered a standard package, including a glucose level, erythrocyte sedimentation rate, hemoglobin level and TSH, to general practitioners to screen for some common causes of fatigue in patients, were similar to the results of the other laboratories in the historical iodine deficient area. Moreover, they were also similar to the results of these laboratories when subjects in whom only TSH and/or FT_4 levels were measured were not excluded (data not shown). Due to the very large number of measurements, the effect of the few subjects with thyroid disease who might have been undesirably included despite our selection of combined measurements of glucose, hemoglobin and TSH, is probably negligible.

In conclusion, this study shows that there are differences in the relation between thyroid function and age between populations with differences in iodine intake in the past, despite an adequate iodine status at present. The negative association between TSH and age in elderly is only present in areas with a history of (mild) iodine deficiency. This raises the question whether, besides age and race, the present but also historical iodine status of a population should be taken into account when establishing the reference limits of TSH and FT_4.

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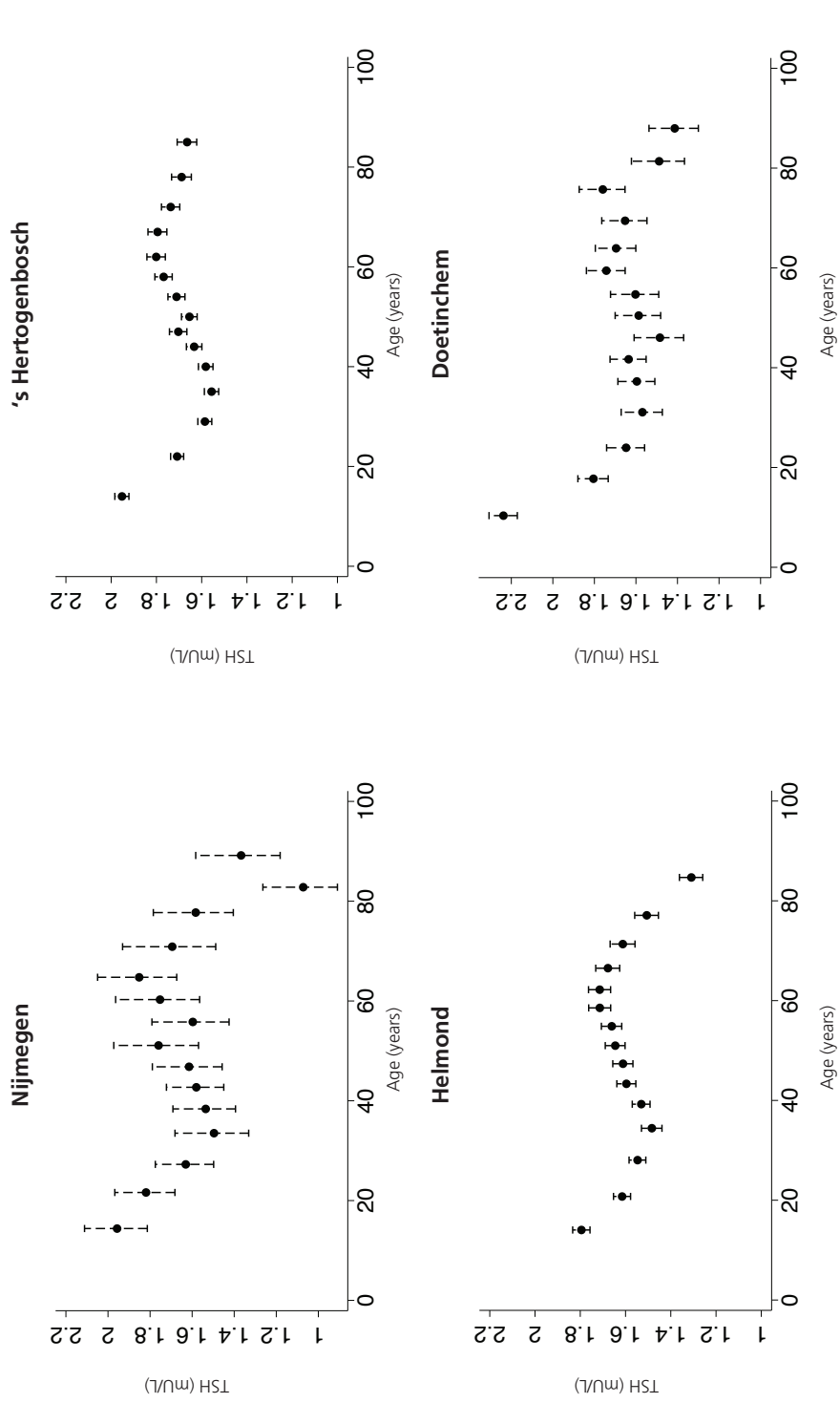
Supplementary Table 1.
Details of the participating laboratories and the laboratory methods.

	Number of TSH measurements	Number of FT_4 measurements	Assay TSH and reference values	Assay FT_4 and reference values
Nijmegen	3574	697	Roche Modular E170, sandwich ECLIA 0.27-4.2	Roche Modular E170, competitive ECLIA 10-23 pmol/L
's-Hertogenbosch	90,003	15,077	Until 2009: Roche Modular E170, sandwich ECLIA 0.35 – 4.0 mU/l 2009-2011: Siemens Dimension Vista, ECLIA based on LOCI 0.35 – 4.0 mU/l	Until 2009: Roche Modular E170, competitive ECLIA 12.0 – 22.0 pmol/l 2009-2011: Siemens Dimension Vista, ECLIA based on LOCI 10.0 – 19.0 pmol/l
Helmond	51,269	5295	Beckman Coulter, CLIA DxI800 0.25-3.1 mU/L	Beckman Coulter, LIA DxI800 7.8-16.0 pmol/L
Doetinchem	15,631	2820	Roche E-170 Cobas 6000, sandwich ECLIA 0.4-4 mU/L	Roche E-170 Cobas 6000, competitive ECLIA 10.0-24.0 pmol/L
Breda	17,597	2408	Until 2009: Siemens Immulite 2000, two side LEIMA 2009-2011 Roche Cobas 6000, two side LEIMA 0.4-4.0 mU/L	Until 2009: Siemens Immulite 2000, competitive LEIMA 2009-2011 Roche Cobas 6000, competitive LEIMA 9-23 pmol/L
Haarlem	6820	4010	Roche Modular E170, sandwich ECLIA 0.28-4.20 mU/L	Roche Modular E170, competitive ECLIA 12.0-22.0 pmol/L
Amsterdam	137,686	69,227	Roche Modular E170, sandwich ECLIA 0.28-4.20 mU/L	Roche Modular E170, competitive ECLIA 12.0-22.0 pmol/L
Leiden	8222	4406	Siemens Immulite 2000, sandwich CLIA 0.4-4.0 mU/L	Siemens Immulite 2000, competitive CLIA 10.3-24.5 pmol/L

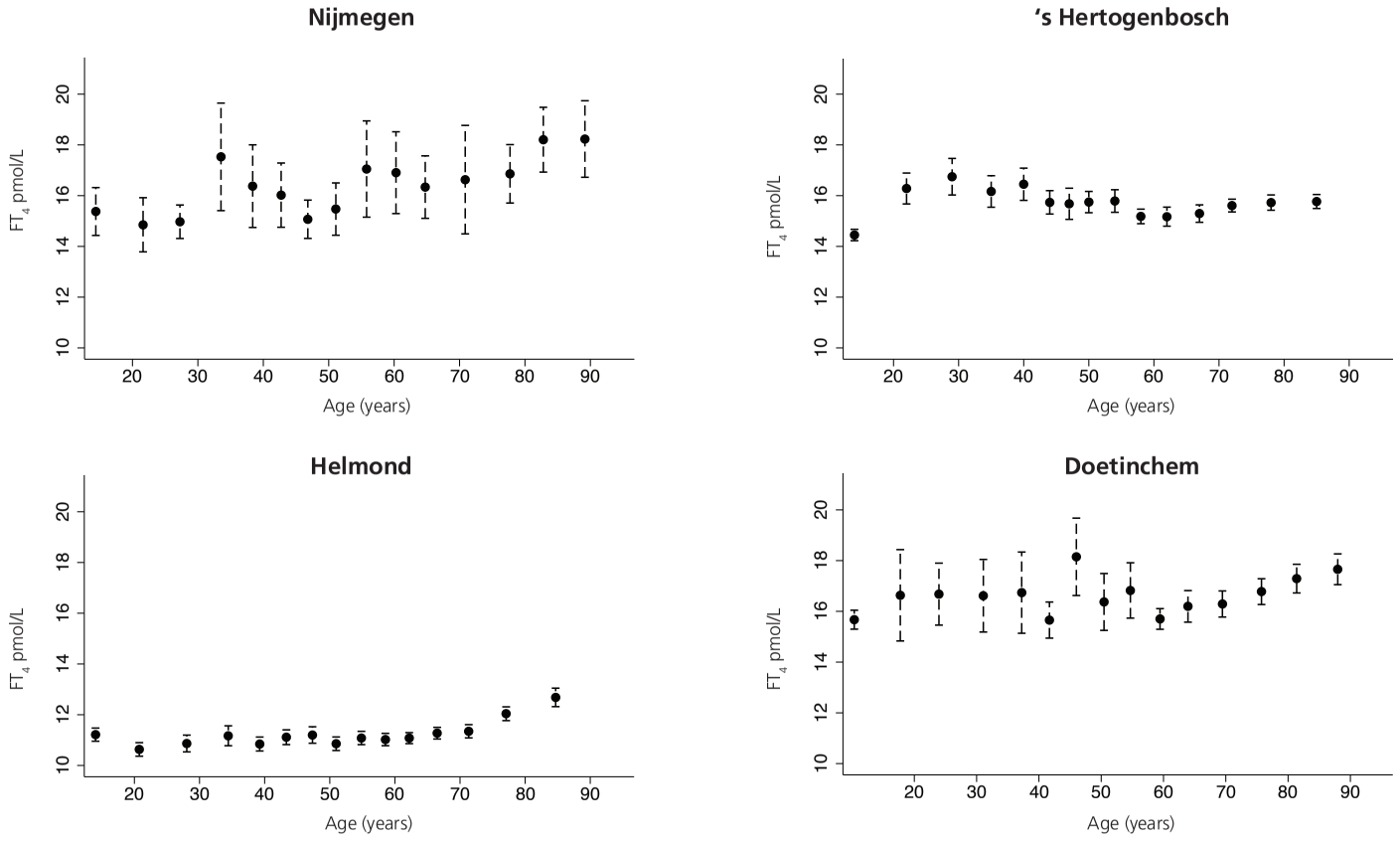
ECLIA= Electro Chemiluminescence Immuno Assay; LOCI= Luminescent Oxygen Channeling Immunoassay; CLIA= Chemiluminescence Immuno Assay; LEIMA= Luminescent Enzyme Immuno Assay



Supplementary Figure 1.
Geometric means (95% CI) of serum TSH levels by age. CI, confidence interval; TSH, thyrotropin.

Haarlem
Leiden
Breda
Amsterdam
TSH (mU/L)
Age (years)
1
1.2
1.4
1.6
1.8
2
2.2
0
20
40
60
80
100

Supplementary Figure 2.
Means (95% CI) of serum FT_4 levels by age. CI, confidence interval; FT_4, free thyroxine.



Haarlem
Leiden
Breda
Amsterdam
FT4 pmol/L
Age (years)
10
12
14
16
18
20
30
40
50
60
70
80
90

CHAPTER 8

General discussion and future perspectives

Results of this thesis in perspective

The aim of this thesis was to investigate short and long term effects of subtle differences in thyroid function and iodine status in the general population. We have shown that even within the normal range of serum TSH and FT_4 levels, fatigue was more severe in subjects with high-normal thyroid function. We found no association between thyroid function within the normal range and presence of depression. Both a high-normal serum TSH and a high-normal serum FT_4 were associated with mortality in older, not in younger subjects. We have also shown that in populations with a mild iodine deficiency in the past, there is an ongoing decrease of serum TSH over time leading to a negative association between serum TSH and age, despite an adequate iodine status at present. Therefore, the main conclusions of this thesis are that:

1) subtle differences of thyroid function within the normal range are associated with short term effects (symptoms) and long term effects (mortality),

2) mild iodine deficiency in the past can affect thyroid function at present.

As summarized in the introduction of this thesis (Chapter 1), numerous previous studies examined the relationship between thyroid function within the normal range and cardiovascular risk factors, morbidity and mortality.[1-43] These studies reported discrepant results on virtually every clinical outcome. Some studies of this thesis also provide results that are in contrast with other studies. For example, although we could not find an association between thyroid function within the normal range and depressive symptoms in middle-aged persons (Chapter 3), a recently published population-based cohort study did find that elderly persons with low-normal TSH levels have more depressive symptoms.[44] These discrepant results can (partially) be explained by the different characteristics of the study populations. Our study comprised middle-aged subjects, whereas the population of the study of Medici et al. consisted of elderly only. As we have shown in this thesis, age also has a major impact on the relationship between thyroid function and mortality (Chapter 4). When interpreting studies investigating the relationship between thyroid function within the normal range and clinical outcomes, the age of the study population should be taken into account as several associations between thyroid function and clinical outcomes are only found in certain age groups (often the elderly). Also gender is an important factor to consider. For example, recently Leader et al. found that serum TSH levels within the lower normal range are associated with an increased risk of hip fractures in euthyroid elderly women, but not in men.[45]

Besides age and gender, the present or historical iodine status of the study population could be an important factor to consider when interpreting studies investigating the relationship between thyroid function within the normal range and clinical outcomes. As we have shown in Chapter 6 and 7, a mild iodine deficiency in the past can affect thyroid function at present. One could hypothesize that the association between thyroid function within the normal range and clinical outcomes differs between two populations with a different iodine status. For example, the association between a low-normal serum TSH level and atrial fibrillation that was found in a population, consisting of elderly living in a historically iodine sufficient area[26] might be absent in a population with a mild iodine deficiency at present or in the past, in which the average serum TSH decreases with age.

In conclusion, the fact that the numerous studies investigating the relationship between thyroid function within the normal range and clinical outcomes provide discrepant results can (partially) be explained by different characteristics of the study populations.

Perspective on the 'optimal' TSH level

Thyroid function is generally considered as having a U-shaped relationship with adverse clinical outcomes at hyperthyroid or hypothyroid extremes. When considering the studies in this thesis and the previous studies regarding associations between thyroid function within the normal range and medical conditions, it seems that for individual symptoms/conditions there are no U-shaped associations within the normal range. Fatigue, depressive symptoms, atrial fibrillation, osteoporosis and dementia are associated with high-normal thyroid function. By contrast, less favorable lipid concentrations, insulin resistance, adiposity, high blood pressure, chronic kidney disease and myocardial infarction are associated with low-normal thyroid function.[1-43, 46] We conclude that there is no single serum TSH level within the reference range that is optimal. There seems to be a different optimal serum TSH level for different medical conditions. Moreover, this optimal serum TSH level may differ between populations with different patient characteristics like age, gender and iodine status.

Perspective on reference values of serum TSH

For some time, the reference values of serum TSH have been debated. Especially the upper limit of the reference range has been questioned. As noted in the introduction of this thesis (Chapter 1), reasons to question the present reference range of TSH are the high proportion of people whose serum TSH is less than 2.5 mIU/L, the higher prevalence of thyroid autoantibodies in people with serum TSH higher than 2.5 mIU/L and the observation that people with serum TSH between 2.5 and 4.5 mIU/L have an increased risk of progression to overt hypothyroidism.[47, 48] However, since about 20% of the subjects without known thyroid disease, without thyroid autoantibodies and with normal thyroid ultrasound has a TSH level between 2.5 mIU/L and 4.5 mIU/L and no firm evidence is available that lowering the upper limit of normal will provide any short- or long-term benefit for subjects, the upper limit of the reference range of TSH remained unchanged.[49-51]

Currently, the reference range of TSH values is based on the 2.5th and 97.5th percentile of the population. Another, more logical, approach for assessing the reference range of serum TSH might be to base the reference limits on clinical outcomes. That is one of the reasons why studies investigating the relationship between thyroid function within the normal range and clinical outcomes are of major interest. The results of this thesis do not support the suggestion of lowering the upper limit of the reference range of serum TSH. Subjects with a serum TSH level in the upper range of normal did not report more symptoms like fatigue or depressive symptoms (Chapter 2 and 3). On the contrary, subjects with a low-normal TSH experienced more fatigue. A high-normal serum TSH value was not associated with mortality in the general population (Chapter 4), with the exception of the very elderly. As described above, previous studies showed that there are several harmful clinical outcomes associated with a low-normal serum TSH whereas several other negative outcomes are associated with a high-normal serum TSH value.[1-43, 46] Given these discrepant results, we think that lowering the limit of serum TSH level based on clinical outcomes is not justified.

In contrast with the suggestion of lowering the upper limit of the reference range of serum TSH in the general population, it has been suggested to increase the TSH upper reference limit in the elderly. Reason for this would be the fact that in several population-based studies, serum TSH is positively associated with age.[52-58] These studies were performed in populations with normal or high iodine intake. However, as shown by the studies described in Chapter 6 and 7 of this thesis, we found a negative relationship between TSH and age and an ongoing decrease of TSH over time in a population with iodine deficiency in the past. These results are in line with previous studies, reporting a negative relationship between serum TSH levels and age in iodine

deficient populations.[59-62] When using the 2.5th and 97.5th percentile to assess the reference range of serum TSH level, the suggestion of increasing the upper limit of the reference range in elderly would not be appropriate for populations with a present or historical iodine deficiency.

When basing the reference limits of elderly on clinical outcomes, the results of the Leiden 85+ study, that reported a protective effect of high-normal TSH values in elderly, would support increasing the upper limit of the reference range of TSH. Recently, Cappola et al. also reported that several negative clinical outcomes, including mortality, were associated with a low serum TSH within the normal range in elderly.[46] However, the study presented in Chapter 4 of this thesis and two other previous studies could not confirm these findings.[39, 55] By contrast, in our study, a high-normal TSH level was associated with mortality in the oldest elderly. Given these discrepant results, we think that the upper limit of the reference range of serum TSH in elderly for the moment should remain unchanged, pending further research that provides more clarity on this subject.

In contrast with the discrepant findings regarding the association between serum TSH and mortality in elderly, the association between a high-normal serum FT_4 and mortality in elderly is a consistent finding in all studies.[38, 39, 46, 55, 63] The reason why a high-normal serum FT_4 level is a better predictor for mortality in elderly than a low-normal serum TSH level is not clear. These results might suggest that there is a change of pituitary TSH set point in elderly with higher FT_4 levels not causing the same TSH suppression as in younger individuals. Possible mechanisms for this are an altered pituitary sensitivity for thyroid hormones, a decrease in TSH bioactivity and/or a decreased responsiveness of the thyroid gland to TSH in elderly.

Implications for clinical practice

This thesis comprises several population-based studies that provide the opportunity to investigate associations between thyroid function and health outcomes without selection bias. However, the question is to what extend one can apply the data of population studies to the individual patient.

An important issue one should take into account when extrapolating data of population studies to the individual patient is the fact that each individual has his/her own set-point of the hypothalamic-pituitary-thyroid axis. The width of the 95% confidence interval of thyroid hormone levels in an individual is approximately half that of the population.[64] The individual set-

point of the hypothalamic-pituitary-thyroid axis is not only determined by environmental factors (such as iodine intake), but also by genetic factors.[64] Therefore, an individual optimal serum TSH level may differ from the average optimal serum TSH level in a population. For example, in a patient suffering from fatigue, his or her optimal serum TSH level can still be in the lower range of normal due to genetic factors, despite the fact that in our population-based study a serum TSH level in the lower range was associated with a higher frequency and severity of fatigue (Chapter 2).

Another issue to keep in mind is that population-based studies are observational and mostly cross-sectional studies. Therefore, no causal relationship or lack of causal relationship between thyroid function and outcomes can be determined. Moreover, we need intervention trials to determine whether a possible intervention results in improvement of the negative health outcomes that are associated with certain serum TSH levels.

Nevertheless, a clinician can keep the results of the population studies in mind while treating patients. The clinician can take into account that there seems to be a different optimal serum TSH level for each medical condition. In clinical practice, it would fit the concept of personalized medicine to consider patient specific factors when deciding what would be the best treatment for the individual patient (i.e. what is the optimal level of serum TSH to aim for). For example, when treating a fatigued older woman with a medical history of osteoporosis with thyroid hormones, it seems reasonable to aim for a serum TSH level in the middle or upper range of normal, given the association between a low-normal serum TSH level and both fatigue (Chapter 2) and an increased risk of hip fracture in older women in population-based studies.[45] On the other hand, one could aim for a serum TSH level in the lower range in an obese middle aged man with hypercholesterolemia, considering the positive association between a high-normal serum TSH level and adiposity and lipid levels.

A common but difficult problem in clinical practice for clinicians as well as for patients is the persistence of symptoms (such as fatigue) during treatment of thyroid dysfunction, despite optimal biochemical control (i.e. both serum TSH and FT_4 levels within the normal range). As we have shown in our study described in Chapter 2 of this thesis, subjects with previously known thyroid disease and thyroid hormone levels within the normal range during treatment reported fatigue substantially more often than euthyroid subjects without known thyroid disease. It is important for clinicians to acknowledge the patients complaints and to explain that this is not rare during treatment of thyroid dysfunction. The reasons for this problem are unclear but subtle abnormalities in thyroid hormone levels should be considered. Substitution therapy with thyroid hormones is perhaps not as optimal as natural secretion of thyroid hormones, strictly

regulated by the hypothalamic-pituitary-thyroid axis. For instance, it is impossible to mimic the subtle variations in thyroid function due to biological variation (e.g. circadian or seasonal variation) with substitution therapy. Also, the treatment goal of the clinician is usually a serum TSH and FT_4 within the normal range. However, as explained above, the individual set-point of the hypothalamic-pituitary-thyroid axis has a small 95% confidence interval. It is possible that a serum level of TSH within the normal range of the population is not within the normal range of the individual patient. This might cause inadequate substitution with thyroid hormones, causing persistent symptoms.

Besides the possible imperfections of substitution therapy with thyroid hormones as a cause of persistent symptoms, the possibility that thyroid dysfunction is not the cause of the symptoms should be considered. In the study described in Chapter 2, we have shown that the prevalence of fatigue in subjects with thyroid dysfunction but without a previously known medical history of thyroid disease was only slightly higher than the prevalence of fatigue in euthyroid subjects. Because of the high prevalence of both fatigue and thyroid dysfunction in the general population, it is likely that many patients who seek medical care because of fatigue, happen to be tired and by coincidence also have thyroid dysfunction, without any causal relationship. Patients with fatigue are more likely to have their thyroid status tested by the general practitioner and (subtle) thyroid dysfunction will be found more often, despite the absence of a causal relationship (confounding by indication). Clinicians should be aware of this possibility. This issue can be discussed with the patient and, if indicated, further diagnostic investigations or other treatment options can be considered.

This thesis comprises two studies regarding the effect of current and historical iodine status on the relationship between thyroid function and age. To assess the iodine status of a population, measurements of urinary iodine, thyroid size, serum TSH and thyroglobulin are used. The median iodine concentration of series of single urine samples is the most widely used measurement.[65, 66] Urinary iodine excretion indicates current iodine nutrition. Neonatal serum TSH screening also provides information on the iodine status of a population. In regions of iodine deficiency, the frequency of supranormal TSH concentrations in neonates is higher than in iodine sufficient areas and this roughly correlates with the severity of iodine deficiency.[67] Thyroid size and serum thyroglobulin concentration reflect iodine nutrition over a period of months or years.[68, 69] In the studies described in Chapter 6 and 7 of this thesis, we show that the relationship between TSH and age is also an indicator of the iodine status of a population. In populations with a mild iodine deficiency in the past, there is an ongoing decrease of serum TSH over time leading to a negative association between serum TSH and age. Since the population of this study has been considered to be iodine sufficient for more than 30 years, we

can conclude that the relationship between TSH and age reflects iodine nutrition over a period of at least several decades.

As part of preventive healthcare, monitoring the iodine status and maintaining an optimal iodine intake is very important to prevent brain damage in newborns and thyroid function disorders at all ages. Besides measuring urine iodine concentrations, neonatal serum TSH, thyroglobulin and thyroid size, one could evaluate the relationship between TSH and age in a population as an additional indicator of iodine deficiency at present or in the past.

Recommendations for further research

As summarized previously in this thesis, there are several cross-sectional population-based studies investigating associations between mild thyroid dysfunction or thyroid function within the normal range and clinical outcomes. However, the benefits and risks of treatment of subtle thyroid dysfunction are still a matter of debate. Only a few randomised controlled trials have been performed in patients with subclinical thyroid disease (mainly in patients with subclinical hypothyroidism) and these trials reported discrepant results with low quality of evidence due to the small numbers of included patients and the small effect sizes.[70-74] Therefore, large prospective randomized controlled intervention trials are required to assess the benefits and risks of treatment of subtle thyroid dysfunction.

We concluded in this thesis that there seems to be a different optimal TSH level for each medical condition. This optimal serum TSH level may differ between populations with different patient characteristics. Therefore, it would be interesting to assess the target level of TSH during treatment in different pre-defined subgroups of patients (classified according to age, gender, medical history etc.) For example, in patients with subclinical hypothyroidism experiencing fatigue, one could investigate in a prospective randomized trial the effect of thyroid hormone replacement therapy on fatigue using different target levels of serum TSH: high-normal TSH target levels versus low-normal TSH target levels versus placebo. The outcome of such a study (i.e. the optimal TSH level) may be different from the outcome of a similar study investigating the effect on for example lipid levels. Also, the outcome may be different in groups with different patient characteristics, such as different age groups. Especially the subgroup of the elderly is of major interest, since some associations between thyroid function within the normal range and negative outcomes are different in elderly in comparison to the general population (Chapter 4) and serum thyroid parameters change during aging (Chapter 6 and 7). However,

to perform studies that investigate each symptom and medical condition separately in each subgroup with different patient characteristics would be a practical challenge. And even if one would be able to perform such studies and an optimal serum TSH would be established for a certain patient group, extrapolating data of these studies to the individual patient would be difficult since every patient has its own set-point of the hypothalamic-pituitary-thyroid axis, in part determined by genetic factors.

One of the striking results of this thesis is the finding that subjects with previously known thyroid disease but thyroid hormones levels within the normal range during treatment reported fatigue substantially more often in comparison to euthyroid subjects without known thyroid disease (Chapter 2). As already mentioned, in clinical practice this is a common and difficult problem. The cause of persistent symptoms after normalization of serum TSH in patients with thyroid disease should be clarified and further treatment options can be explored. For example, one could investigate the effect of changing the target of serum TSH levels, the beneficial and/or harmful effects of treatment with combination therapy of levothyroxine and liothyronine (T_3) or the efficacy of other treatment options like cognitive behaviour therapy or rehabilitation programs.

In Chapter 6 and 7 of this thesis we have shown that in populations with a mild iodine deficiency in the past, there is an ongoing decrease of serum TSH over time leading to a negative association between serum TSH and age, despite an adequate iodine status at present. The negative association between TSH and age was only present in elderly (60 years or older) who had been exposed to iodine deficiency in the past. In younger subjects (aged 35-60 years old), TSH was positively associated with age. We hypothesized that this difference in relationship is caused by the fact that the younger subjects are not exposed to iodine deficiency in the past. Therefore, the younger subjects are less likely to have developed growth and autonomous function of the thyroid. To test this hypothesis, it would be interesting to study the relationship between TSH and age again in the same area in several decades. Probably we would find a positive association between TSH and age in all age groups, in line with the results of the population studies performed in (historical) iodine sufficient areas. This finding would be an extra argument to consider this population as iodine sufficient.

As already mentioned, monitoring the iodine status and maintaining an optimal iodine intake is very important to prevent brain damage in newborns and thyroid function disorders at all ages. The new finding that mild iodine deficiency in the past causes changes in thyroid function to date stresses the importance of regular monitoring of the iodine status of a population in order to maintain an optimal iodine intake.

Case: a personal reflection

This thesis started with a clinical vignette presenting the case of a 74-year-old woman, living in an iodine deficient area, with symptoms of fatigue, weight gain and cold intolerance and a TSH level of 3.8 mIU/L (reference range 0.4-4.0 mIU/L) before treatment with thyroid hormone replacement therapy. After the initiation of thyroid hormone replacement therapy, fatigue persisted despite a TSH level of 2.6 mIU/L. This case raises several questions, as formulated in the introduction chapter (Chapter 1). When taking the results of this thesis and those of previous studies into account, the case can be re-evaluated as follows:

Before the start of my PhD project, I would have classified this patient without any hesitation or consideration as having a normal thyroid function. However, the results of this thesis yielded in a more nuanced and differentiated view on this topic. One argument that would favor the opinion that the thyroid function is abnormal in this case is that, as outlined before, each individual has its own setpoint of the hypothalamic-pituitary-thyroid axis and it is possible that this value of serum TSH of 3.8 mIU/L within the reference range is not normal for this individual patient, given also the presence of thyroid peroxidase antibodies. Moreover, this patient has presumably been exposed to iodine deficiency and the average TSH level decreases with age in populations with iodine deficiency. Therefore, the TSH level in the upper range of normal may be inadequately high for this patient. However, there are no intervention trials that show convincing evidence that treatment of such subtle thyroid dysfunction results in relief of symptoms. Therefore, national and international guidelines advise against prescribing thyroid hormone replacement therapy in patients with TSH levels within the normal range.

The results of this thesis did change my clinical practice in patients with persistent symptoms during treatment with thyroid hormone replacement therapy. Nowadays, I explain to patients that this is a common problem during treatment and that several factors may play a role. I discuss that thyroid hormone replacement therapy is perhaps not as optimal as natural secretion of thyroid hormones, strictly regulated by the hypothalamic-pituitary-thyroid axis with an individual set-point with a small margin. Subsequently, I would try to find the individual optimal TSH level (within the normal range) for the patient, titrating according to the symptoms, taking into account the characteristics of the patient. In addition, I now discuss with patients the fact that fatigue and mild thyroid dysfunction frequently are both present in patients without any causal relationship and if indicated I consider further diagnostic investigations or other treatment options (like cognitive behavior therapy or a rehabilitation program).

Conclusion

In conclusion, the results of this thesis show that subtle differences of thyroid function within the normal range are associated with short-term effects (symptoms) and long-term effects (mortality) and that mild iodine deficiency in the past can affect thyroid function at present. The results of this thesis do not support the suggestion of changing the limits of the reference range of serum TSH. Within the normal range, there seems to be a different optimal serum TSH level for each symptom and medical condition. This optimal serum TSH level may differ between populations with different patient characteristics, like age or iodine status. A clinician can take this into account when aiming for the optimal level of serum TSH during thyroid hormone replacement therapy.

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CHAPTER 9

Summary

Chapter 1 provides an overview of the function of thyroid hormones, the effects of thyroid dysfunction, the importance of an adequate iodine intake and the effects of iodine deficiency. The aim of this thesis is to investigate short and long term effects of subtle differences in thyroid function and iodine status in the general population. The studies presented in this thesis are introduced.

In **Chapter 2**, a study investigating the association between thyroid function within the normal range and fatigue is presented. A total of 5897 participants of the Nijmegen Biomedical Study filled out a questionnaire and serum thyroid stimulating hormone (TSH) and free thyroxine (FT_4) were measured. Fatigue was evaluated by the RAND-36 vitality subscale and the Shortened Fatigue Questionnaire (SFQ). Euthyroid subjects with a serum TSH level of 0.4-1.0 mIU/L had a lower RAND-36 vitality score (65.2 vs. 66.8; regression coefficient (RC) -1.6, 95% confidence interval (CI) -2.6 to -0.5) and a higher SFQ score (11.7 vs. 11.0; RC 0.6, 95% CI 0.2-1.0) than those with a TSH of 1.0-2.0 mIU/L. Those with a serum FT_4 of 18.5-22 pmol/L reported fatigue more often (52.5% vs. 33.3%; RR 1.4, 95% CI 1.0-1.9), had a lower RAND-36 vitality score (61.7 vs. 66.6; RC -4.4, 95% CI -8.1 to -0.6) and a higher SFQ score (13.2 vs. 11.0; RC 1.9, 95% CI 0.4-3.3) than subjects with a FT_4 level of 11.5-15 pmol/L. In comparison to euthyroid subjects without known thyroid disease, euthyroid subjects with previously known thyroid disease reported fatigue more often (52.3% vs. 34.0%; relative risk (RR) 1.3, 95% CI 1.0-1.5), had a lower RAND-36 vitality score (61.4 vs. 66.3; RC -2.9, 95% CI -5.3 to -0.6) and a higher SFQ score (13.7 vs. 11.1; RC 1.4, 95% CI 0.5-2.3). We concluded that within the normal range of serum TSH and FT_4, fatigue seemed more severe in subjects with low-normal TSH levels and high-normal FT_4 levels than middle-normal TSH and FT_4 levels, although the effect was small. Subjects with a history of thyroid disease, but with normal serum TSH and FT_4 concentrations, experienced more fatigue than the general population.

Chapter 3 describes a study investigating whether thyroid function and thyroid peroxidase antibodies (TPOAb) are associated with depression, when using both state and trait parameters of depression. In 1125 participants of the Nijmegen Biomedical Study serum TSH, FT_4 and TPOAb were measured twice. The Beck Depression Inventory 1A (BDI), a self-reported lifetime diagnosis of depression and the neuroticism scale of the Eysenck Personality Questionnaire Revised Short Scale (EPQ-RSS) were used to evaluate the presence of state and trait features of depression. We found no association between serum TSH and FT_4 levels and BDI score, current depression, lifetime diagnosis of depression and EPQ-RSS neuroticism score. Subjects with TPOAb had higher EPQ-RSS neuroticism scores in comparison to subjects without TPOAb, mean score 4.1 versus 3.2 (RC 0.70, 95% CI 0.1-1.3). The prevalence of a lifetime diagnosis of depression was higher in subjects with positive TPOAb in comparison to participants

without TPOAb: 24.2% versus 16.7% (RR 1.4, 95% CI 1.0-2.1). We concluded that there was no association between thyroid function and depression. However, TPOAb were positively associated with trait markers of depression. The presence of TPOAb may be a vulnerability marker for depression.

Chapter 4 reports a study investigating the influence of age on the relationship between thyroid function and mortality. In 5816 participants of the Nijmegen Biomedical Study without previously known thyroid disease, serum TSH, FT_4 and TPOAb were measured in 2002-2003. The number of deaths was established in 2012 (median follow up time 9.4 years). Subclinical thyrotoxicosis was associated with mortality in subjects aged <65 years (hazard ratio (HR) 2.5, 95% CI 1.1-5.7), but not in subjects aged >65 years. As for thyroid function within the normal range: in the 493 participants aged 80 years or older, a serum FT_4 level in the high-normal range (18.5-22 pmol/l) was associated with a higher mortality in comparison with FT_4 levels in the middle range (11.5-15.0 pmol/l): HR 1.7 (95% CI 1.0-2.9). In these elderly, TSH levels within the high-normal range (3.0-4.0 mIU/l) were also associated with a higher mortality in comparison with TSH levels within the middle range (1.0-2.0 mIU/l): HR 1.8 (95% CI 1.0-3.1). In summary, we demonstrated that the relationship between thyroid function and mortality differs according to age. This finding might (partially) explain the discrepant results of previous studies examining the relationship between thyroid function and mortality in different age groups.

In **Chapter 5** we examined the within-individual variability of serum TSH and FT_4 measurements several years apart in different study cohorts and we determined the magnitude of the underestimation of the association between thyroid function and disease rates in population-based studies using only one baseline measurement. Due to regression dilution bias, variation in thyroid function can blur the association between thyroid function and the outcomes of interest. We used a pair of measurements of serum TSH and FT_4 levels of subjects of the Nijmegen Biomedical Study (NBS) and the Rotterdam Study (RS) to calculate the regression dilution ratio (RDR) with a nonparametric method, the MacMahon's method. Risk estimates could be corrected for regression dilution bias by dividing them by the RDR. The RDRs of serum TSH in the NBS and RS were 0.74 and 0.78, respectively. The RDRs of serum TSH were similar for women and men. The RDR of serum FT_4 in subjects in the NBS was 0.77. We concluded that the relationship between thyroid function and disease rates is underestimated by studies using only one measurement of TSH and FT_4. The true association will be about 33% (1/0.75) higher for studies with a follow-up time of 2-4 years.

In **Chapter 6** a longitudinal study is presented, investigating whether the decrease of serum TSH and the increase of serum FT_4 with age, previously found in a cross sectional survey in a

previously iodine deficient area, is an ongoing process apparent also in longitudinal analyses and whether it reflects an actual iodine deficiency or an iodine deficiency in the past. In 980 participants of the Nijmegen Biomedical Study, we measured serum TSH, FT_4, total T_3, peroxidase antibodies and the urine iodine and creatinine concentration 4 years after our initial survey of thyroid function in which we reported a negative association between serum TSH and age. Within 4 years, serum TSH decreased with 5.4% (95% CI 2,5%-8,3%) and serum FT_4 increased with 3.7% (95% CI 2.9%-4.6%). The median urinary iodine concentration of the population was 130 µg/L. The estimated 24-hour iodine excretion was not associated with serum TSH, T_3, change of TSH or FT_4 over time or with the presence of TPOAb. Only FT_4 appeared to be somewhat higher at lower urine iodine levels: a 1.01% (95% CI 0.17-1.84) higher FT_4 for each lower iodine quintile. In conclusion, we found an ongoing decrease of serum TSH and increase of serum FT_4 in a previously iodine insufficient population, despite the adequate iodine status at present. This suggests that low iodine intake at young age leads to thyroid autonomy (and a tendency to hyperthyroidism) that persists despite normal iodine intake later in life.

Chapter 7 reports a study, investigating whether there are current differences in the relationship between thyroid function and age in relation to differences in iodine intake in the past. Eight medical laboratories in several regions of the Netherlands, which are all iodine sufficient at present, but with a difference in iodine status in the past, provided the results of all serum TSH and FT_4 measurements performed from 2006 until 2011, resulting in 330,802 TSH and 103,940 FT_4 measurements. The negative association between TSH and age in elderly was only present in areas with a historically iodine deficiency (RC -0.008, 95% CI -0.009 to -0.007). In the historical iodine sufficient population, TSH showed no obvious increase or decrease with age. In both the historically iodine sufficient and deficient populations, serum FT_4 levels were positively associated with age in elderly (RC 0.009, 95% CI 0.008-0.010 and RC 0.008, 95% CI 0.007-0.010 respectively). We therefore conclude that there are differences in relationship between thyroid function and age between populations with differences in iodine intake in the past, despite an adequate iodine status at present.

Chapter 8 is a general discussion, that puts the main findings of this thesis in perspective. Additionally, a perspective on the reference values of serum TSH is given and the implications of the results of this thesis on clinical practice are discussed. Finally, recommendations for future research are made.

CHAPTER 10

Samenvatting

List of Publications

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Samenvatting

Hoofdstuk 1 geeft een overzicht van de werking van schildklierhormonen, de gevolgen van een gestoorde schildklierfunctie, het belang van een adequate jodiuminname en de gevolgen van jodiumdeficiëntie. Het doel van dit proefschrift is om de korte en lange termijn gevolgen van subtiele verschillen in schildklierfunctie en jodium status in de algemene bevolking te onderzoeken. De studies van dit proefschrift worden geïntroduceerd.

In **Hoofdstuk 2** beschrijven wij de associatie tussen schildklierfunctie en moeheid. In 2002-2003 vulden 5.897 deelnemers van de Nijmegen Biomedische Studie een vragenlijst in en werd bij hen thyroid stimulerend hormoon (TSH) en vrij thyroxine (FT_4) bepaald in het serum. Moeheid werd geëvalueerd door de subschaal vitaliteit van de RAND-36 en de Shortened Fatigue Questionnaire (SFQ). Euthyreote deelnemers met een serum TSH van 0.4-1.0 mE/L hadden een lagere RAND-36 vitaliteitsscore (65.2 vs. 66.8; regressie coëfficiënt (RC) -1.6, 95% confidence interval (CI) -2.6 tot -0.5) en een hogere SFQ score (11.7 vs. 11.0; RC 0.6, 95% CI 0.2-1.0) dan de deelnemers met een serum TSH van 1.0-2.0 mE/L. Deelnemers met een serum FT_4 tussen 18.5-22.0 pmol/L gaven vaker aan vermoeid te zijn (52.5% vs. 33.3%; relatieve risico (RR) 1.4, 95% CI 1.0-1.9), hadden een lagere RAND-36 vitaliteitsscore (61.7 vs. 66.6; RC -4.4, 95% CI -8.1 tot -0.6) en een hogere SFQ score (13.2 vs. 11.0; RC 1.9, 95% CI 0.4-3.3) dan mensen met een serum FT_4 tussen 11.5-15.0 pmol/L. Mensen met een schildklieraandoening in de voorgeschiedenis maar momenteel een normaal serum TSH en FT_4 rapporteerden vaker vermoeidheid (52.3% vs. 34.0%; RR 1.3, 95% CI 1.0-1.5), hadden een lagere RAND-36 vitaliteitsscore (61.4 vs. 66.3; RC -2.9, 95% CI -5.3 tot -0.6) en een hogere SFQ score (13.7 vs. 11.1; RC 1.4, 95% CI 0.5-2.3) in vergelijking met euthyreote mensen zonder schildklieraandoening. Wij concludeerden dat binnen het normale gebied van serum TSH en FT_4, moeheid ernstiger was bij mensen met een hoog-normale schildklierfunctie in vergelijking met mensen met een schildklierfunctie in het middelste deel van het normale gebied. Mensen met een schildklieraandoening in de voorgeschiedenis, maar met momenteel normale serum TSH en FT_4 waarden, rapporteerden meer moeheid dan mensen zonder schildklieraandoening.

In **Hoofdstuk 3** beschrijven wij de resultaten van de studie waarbij onderzocht werd of de schildklierfunctie en de aanwezigheid van anti-TPO antistoffen (TPOAb) geassocieerd zijn met depressie, gebruikmakend van zowel state als trait parameters van depressie. Bij 1.125 deelnemers van de Nijmegen Biomedische Studie werden serum TSH, FT_4 en TPOAb twee keer bepaald. De Beck Depression Inventory (BDI), de aanwezigheid van een depressie in de voorgeschiedenis en de neuroticisme schaal van de Eysenck Personality Questionnaire Revised Short Scale (EPQ-RSS) werden gebruikt om de state en trait kenmerken van depressie te

evalueren. Er werd geen associatie gevonden tussen de serum TSH en FT_4 waarden en de BDI score, de aanwezigheid van een huidige depressie, het ooit doorgemaakt hebben van een depressie en de EPQ-RSS neuroticisme score. Mensen met TPOAb hadden een hogere EPQ-RSS neuroticisme score in vergelijking met mensen zonder TPOAb (gemiddelde score 4.1 versus 3.2 (RC 0.70, 95% CI 0.1-1.3)). De prevalentie van een doorgemaakte depressie was hoger bij mensen met TPOAb dan bij mensen zonder TPOAb: 24.2% versus 16.7% (RR 1.4, 95% CI 1.0-2.1). Samenvattend vonden wij geen associatie tussen schildklierfunctie en depressie. Wel is de aanwezigheid van TPOAb geassocieerd met trait parameters van depressie. Mogelijk is de aanwezigheid van TPOAb een marker voor kwetsbaarheid voor depressie.

Hoofdstuk 4 rapporteert een studie die de invloed van leeftijd op de relatie tussen schildklierfunctie en mortaliteit onderzocht. Bij 5.816 deelnemers van de Nijmegen Biomedische Studie werden TSH, FT_4 en TPOAb in serum gemeten in 2002-2003. Het aantal overledenen werd vastgesteld in 2012 (mediane follow up duur 9.4 jaar). Subklinische hyperthyreoidie was geassocieerd met mortaliteit bij mensen jonger dan 65 jaar (hazard ratio (HR) 2.5, 95% CI 1.1-5.7), maar niet bij mensen ouder dan 65 jaar. Subklinische en manifeste hypothyreoidie waren niet geassocieerd met mortaliteit. Ten aanzien van de schildklierfunctie binnen het normale gebied werden de volgende resultaten gevonden: bij de 493 deelnemers die ouder waren dan 80 jaar was een hoog-normale serum FT_4 waarde van 18.5-22.0 pmol/L geassocieerd met een hogere mortaliteit in vergelijking met een serum FT_4 waarde van 11.5-15.0 pmol/L: HR 1.7 (95% CI 1.0-2.9). Bij ouderen waren ook hoog-normale serum TSH waarden van 3.0-4.0 mE/L geassocieerd met mortaliteit in vergelijking met serum TSH waarden van 1.0-2.0 mE/L: HR 1.8 (95% CI 1.0-3.1). Deze associaties werden niet bij jongeren gevonden. Samenvattend laat deze studie dus zien dat de relatie tussen schildklierfunctie en mortaliteit verschilt tussen verschillende leeftijdscategorieën. Deze bevinding zou een verklaring kunnen zijn voor de discrepante resultaten van de voorgaande onderzoeken die de relatie tussen schildklierfunctie en mortaliteit beschrijven waarbij verschillende populaties met verschillende leeftijdscategorieën zijn onderzocht.

Hoofdstuk 5 vermeldt een onderzoek waarin de intra-individuele variabiliteit van serum TSH en FT_4 werd onderzocht en de mate waarin de associatie tussen schildklierfunctie en symptomen, aandoeningen of mortaliteit daardoor onderschat wordt. Ten gevolge van de regression dilution bias kan variatie in schildklierfunctie de associatie tussen schildklierfunctie en gezondheidsgerelateerde uitkomsten afzwakken. Serum TSH en FT_4 werden enkele jaren na elkaar gemeten in twee verschillende studie cohorten, de Nijmegen Biomedische Studie en de Rotterdam Studie. Hiermee werd de regression dilution ratio (RDR) berekend met een nonparametrische methode, de MacMahon methode. Uitkomstmaten van associaties kunnen

worden gecorrigeerd voor de regression dilution bias door de uitkomstmaat te delen door de RDR. De RDRs van serum TSH in de Nijmegen Biomedische Studie en de Rotterdam Studie waren respectievelijk 0.74 en 0.78. De RDRs van TSH waren vergelijkbaar voor mannen en vrouwen. De RDR van serum FT_4 van deelnemers van de Nijmegen Biomedische Studie was 0.77. Wij concludeerden dat de relatie tussen schildklierfunctie en aandoeningen onderschat wordt door studies die slechts één meting van TSH en FT_4 gebruiken. De daadwerkelijk associatie zal ongeveer met 33% (1/0.75) sterker zijn in studies met een follow up duur van twee tot vier jaar.

In **Hoofdstuk 6** wordt een studie gepresenteerd, waarbij werd onderzocht of de daling van serum TSH en de stijging van serum FT_4 met de leeftijd, eerder aangetoond in een cross-sectionele studie in een gebied met in het verleden een jodiumtekort, een doorlopend proces is dat ook te zien is bij longitudinale analyse. Daarnaast werd bekeken of dit veroorzaakt wordt door een huidig jodiumtekort. In 2002-2003 werden bij deelnemers van de Nijmegen Biomedische Studie serum TSH en FT_4 bepaald. In 2006-2007 werden bij 980 van deze deelnemers nogmaals het serum TSH en FT_4 gemeten, aangevuld met serum T_3 en TPOAb en werden de urine jodium en kreatinine concentraties gemeten. In deze vier jaar daalde het gemiddelde serum TSH met 5.4% (95% CI 2,5%-8,3%) en steeg het FT_4 met 3.7% (95% CI 2.9%-4.6%). De mediane urine jodium concentratie van de populatie was 130 µg/L. De geschatte 24-uurs jodium uitscheiding was niet geassocieerd met serum TSH, T_3, verandering van TSH of FT_4 in de vier jaar of met de aanwezigheid van TPOAb. Alleen het serum FT_4 was hoger bij mensen met een lagere jodiumuitscheiding: een lager jodium quintiel was geassocieerd met een 1.01% hogere serum FT_4 waarde. Concluderend vonden wij in deze longitudinale studie een voortgaande daling van serum TSH en stijging van serum FT_4 in een populatie met in het verleden een jodiumdeficiëntie, ondanks een adequate jodium inname op dit moment. Deze resultaten suggereren dat een lage jodium inname op jonge leeftijd leidt tot autonomie van de schildklier met een neiging tot hyperthyreoidie, die persisteert ondanks een normale jodium intake op latere leeftijd.

In **Hoofdstuk 7** beschrijven wij de verschillen in de relatie tussen schildklierfunctie en leeftijd in populaties met een verschil in jodium inname in het verleden maar met momenteel een adequate jodium inname. Acht laboratoria in verschillende regio's in Nederland leverden de resultaten van alle serum TSH en FT_4 metingen, aangevraagd door huisartsen, verricht van 2006 tot 2011. Dit resulteerde in 330.802 TSH en 103.940 FT_4 metingen. De negatieve associatie tussen serum TSH en leeftijd bij ouderen is alleen aanwezig in gebieden met jodiumdeficiëntie in het verleden (RC -0.008, 95% CI -0.009 tot -0.007). In de populatie met adequate jodiuminname in het verleden werd geen toename of afname van serum TSH met de leeftijd

gevonden. In zowel de historisch jodiumdeficiënte populatie als de historisch jodiumsufficiënte populatie werd een positieve associatie tussen serum FT_4 en leeftijd gevonden bij ouderen (RC 0.009, 95% CI 0.008-0.010 and RC 0.008, 95% CI 0.007-0.010 respectievelijk). Wij concludeerden dat er verschillen zijn in de relatie tussen schildklierfunctie en leeftijd tussen populaties met een verschillende jodiuminname in het verleden, ondanks een huidige adequate jodium inname.

Hoofdstuk 8 is een algemene discussie, waarbij de resultaten van dit proefschrift in perspectief worden geplaatst. Daarnaast beschrijven wij onze visie over de op dit moment gehanteerde normaal waarden van serum TSH. Tevens worden de implicaties van de resultaten van dit proefschrift voor de klinische praktijk besproken. Tenslotte worden er aanbevelingen gedaan voor toekomstig onderzoek.

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Curriculum Vitae

Annenienke van de Ven werd op 1 december 1977 geboren te Boekel. In 1996 behaalde zij haar gymnasiumdiploma (cum laude) aan het Zwijsen College te Veghel. In datzelfde jaar begon ze met de studie geneeskunde aan de toenmalige Katholieke Universiteit Nijmegen, inmiddels Radboudumc. Eind 2002 behaalde zij haar artsexamen.

Hierna verrichtte zij onderzoek op de afdeling Medical Technology Assessment van het Radboudumc. In 2003 begon zij als arts-assistent Geriatrie en Interne Geneeskunde in het Elkerliek Ziekenhuis te Helmond. In 2004 startte zij met de opleiding tot internist en van 2004 tot 2007 werkte zij als AIOS Interne Geneeskunde in het Máxima Medisch Centrum te Veldhoven (opleiders: dr. A.W. van den Wall Bake en dr. R.J. Erdtsieck). In 2007 vervolgde zij haar opleiding in het Radboudumc te Nijmegen (opleiders: prof. dr. J.W.M. van der Meer, prof. dr. J. de Graaf, prof. dr. P.M.J. Stuyt en dr. C.P. Bleeker-Rovers). In 2009 begon zij aan het aandachtsgebied Endocrinologie (opleider: prof. dr. A.R.M.M. Hermus). In 2010 startte zij met het promotieonderzoek, onder begeleiding van prof. dr. M. den Heijer, prof. dr. A.R.M.M. Hermus, prof. dr. F.C.G.J. Sweep en dr. R.T. Netea-Maier. In 2011 voltooide zij de opleiding tot internist. Sindsdien is zij werkzaam als internist-endocrinoloog in het Radboudumc.